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Journal of the Science of Food and Agriculture

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Journal:	*Journal of the Science of Food and Agriculture*
Manuscript ID	Draft
Wiley - Manuscript type:	Short Communication
Date Submitted by the Author:	n/a
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Key Words:	Microemulsion, lemon oil, β-carotene, phostability, shelf life

Microemulsions as delivery systems of lemon oil and β-carotene into beverages: stability test under different light conditions

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Running title

Photostability of beverages containing microemulsions

ABSTRACT

Background

Microemulsions have been proposed as delivery systems for different lipophilic substances in transparent water-based systems. The chemical stability of the delivered compounds is a key factor to broad the application of microemulsions in the food sector. The stability of a model beverage containing microemulsions delivering β-carotene and lemon oil was tested under increasing light intensity up to 6000 lux at 20 °C.

Results Transparent microemulsions resulted physically stable during storage indicating that no coalescence phenomenon occurred. On the contrary, both colour and flavour of the microemulsion degraded as a consequence of limonene and β-carotene oxidation. Kinetic data obtained at increasing light were used to estimate the light dependence of beverage spoilage and the mathematical relationship obtained was used to predict spoilage rate under different light conditions. Finally, a shelf life predictive model was proposed.

Conclusions

Transparent microemulsions can be successfully used to deliver into beverages flavour oil and colorants. However, the photostability of the delivered compounds should be carefully studied to estimate product shelf life. To this aim, the availability of models predicting shelf life as a function of light conditions could largely contribute to speed up the process.

KEYWORDS

Microemulsion, lemon oil, β-carotene, beverages, phostability, shelf life

INTRODUCTION

Microemulsions are thermodynamically stable aqueous dispersions of oil droplets with mean radii in the 2 – 100 nm range[1]. This reduced particle size confers transparent appearance to the system. Microemulsions are usually produced by low energy emulsification methods allowing the spontaneous self-assembly of components under appropriate compositional and environmental conditions[1,2]. Based on these unique features, microemulsions have been proposed in literature as delivery systems for different lipophilic substances (e.g. essential oils, bioactive compounds and drugs) in transparent water-based systems[1].

Recently, Valoppi et al.[3] developed transparent microemulsions stabilized with Tween 80 and delivering large amount of lemon oil, which is one of the major flavor oil used by the beverage industry. These microemulsions were formed by applying the phase inversion temperature method (PIT). The latter relies on coarse emulsion heating above the surfactant PIT, followed by rapid cooling. The abrupt temperature change induces phase inversion thanks to the ability of the surfactant to recovery its original molecular geometry[1]. To obtain a high load of lemon oil in microemulsions, lemon oil was mixed with peanut oil[3]. This strategy allowed to include in transparent systems up to 15 % lemon oil. It was suggested that the presence of an oil rich in long-chain fatty acids beside lemon oil promoted Tween 80 optimum curvature increasing the lemon oil loading capacity.

In the food sector, microemulsions can be considered as stock emulsions containing high levels of the target substances, which are generally lipophilic flavors and/or colorants. Microemulsions are intended for dilution with water to prepare the final beverages[4]. Since mosy microemulsions are formed at limited combinations of their constituents, their dilutability needs being tested to be potentially exploited as delivery systems of lipophilic compounds in transparent beverages. Moreover, being the latter generally packed in see-through materials and displayed on highly enlighten shelves to attract consumers, liposoluble compounds delivered by microemulsions might undergo oxidation during beverage storage. The chemical stability of the delivered flavour/colourants is thus an additional key factor to be considered to broad the application of microemulsions. However, up to now, there is limited information on this aspect.

Three main concomitant events leading to quality depletion during storage of transparent beverages delivering flavour and colors might be expected: i) physical instability with loss of transparency due to oil droplets coalescence and flocculation, ii) color modifications due to pigment depletion and iii) flavour changes due to the degradation of volatile compounds. The challenge is to understand the relative rate of the spoilage events to determine the most critical for the product stability and shelf life.

This work was planned to study the potential exploitation of microemulsions as delivery systems of flavour and colours in transparent beverages. To this aim, microemulsions delivering β-carotene and lemon oil were used to prepare beverages. The latter were submitted to a storage stability test under different light intensity (from 800 to 6000 lux) at 20 °C. Turbidity, β-carotene and limonene content - chosen as indicator of lemon flavour degradation - were monitored during storage under different light conditions to compute kinetic rate constants. Kinetic data obtained at increasing light were used to estimate the light dependence of beverage spoilage with the final aim to develop a predictive stability model exploiting light as acceleration factor[5,6]. The mathematical relationship between beverage spoilage rate and light intensity was used to predict spoilage rate under different light conditions and estimate shelf life.

MATERIALS AND METHODS

Materials

Peanut oil and sucrose were purchased in a local market. Essential lemon oil was kindly provided by Enrico Giotti S.p.A. (Scandicci, Italy). Tween 80, citric acid monohydrate and (*R*)-(+)-limonene were purchased from Sigma-Aldrich (Milan, Italy). NaCl, and sodium benzoate were purchased from Carlo Erba (Milan, Italy). All solutions were prepared using milli-Q water.

Microemulsion preparation

The emulsion was prepared following the methodology proposed by Valoppi et al [3]. The aqueous phase consisted of 30% (w/w) Tween 80 dispersed in NaCl aqueous solutions (0.8 M). The mixture was stirred overnight at 300 rpm in order to dissolve the surfactant before use. The lipid phase was composed of a mixture of lemon oil and peanut oil (3:1) containing 0.6 mg/g of β-carotene.

The aqueous phase and lipid phase were mixed at 600 rpm for 10 min at ambient temperature to form a coarse emulsion. Aliquots of 8 g of the coarse emulsion were transferred into 10 mL vials, sealed and heated for 30 min at 90 °C in a water bath. Samples were then hand shaken until a homogeneous system was obtained and finally cooled in an ice bath until reaching 20 °C.

Beverage preparation

To simulate commercial beverage, an aqueous solution containing citric acid (8.4 g/L; pH 2.1), sucrose (100 g/L) and sodium benzoate (0.8 g/L) use as preservative was prepared. Finally, 5 g/L of the microemulsion was added to the water phase. This concentration was chosen considering the Acceptable Daily Intake (ADI) of Tween 80, as reported by EFSA[7]. Since the surfactant has a maximum ADI of 25 mg/kg die, the calculation was performed considering an average man of 70 kg consuming a maximum amount of 1 L beverage/day[3].

Beverage storage

Aliquots of 8 mL of beverage were introduced into 10 mL capacity clear glass vials and hermetically sealed with butyl septa and metallic caps with air in the headspace (Carlo Erba, Milano, Italy). The vials were stored into an incubator (Climacell 222, MMM Medcenter, Einrichtungen GmbH, Graefling, Germany) at different distances from the SLI Activa-172 fluorescent tubes (34.2 W, Sylvania, SLI Lighting, Raunheim, Germany) positioned vertically in the internal part of the door of the incubator. Irradiance of the fluorescent tubes was 1.199 mW/cm^2 and their emission spectrum was within 250 and 780 nm. Temperature was set at 20 °C and no temperature changes were observed as a consequence of lightning. A control sample was stored inside the incubator in a black box (dark conditions). Accordiing to the enlightening level measured by a portable luminometer (HD-2102.2 Delta Ohm, Padova, Italy), samples resulted to be exposed to 0, 800, 200 and 6000 lx.

Analytical determination

Particle size

Particle size was determined using a Particle Sizer 380 ZLS analyzer (PSS NICOMP Particle Sizing system, Goleta, USA). Before analysis, emulsions were diluted with deionized water in order to avoid multiple scattering effects. Mean particle diameter was expressed as volume weighted mean diameter ± standard deviation (SD).

Turbidity

Aliquots of 2 mL of simulated beverages were introduced in glass cuvette and turbidity was measured using a UV-2501 PC UV-VIS (Shimadzu, Kyoto, Japan) spectrophotometer recording the absorbance at 600 nm.

β-carotene

The changes of sample absorbance at 450 nm (Cary 1E UV/VIS spectrophotometer, Varian, Palo Alto, California) were taken as an index of β-carotene degradation. Data were elaborated as relative percentage of β-carotene as a function of storage time.

Limonene

Limonene was determined by SPME-GC-MS on a GC-17A gas chromatograph, coupled with a QP-5000 mass spectrometer (both from Shimadzu, Kyoto, Japan). Solid-phase microextraction was carried out on 0.5 mL of beverage, at 10 °C, by using a 2 cm 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane fiber (Supelco, Bellefonte, PA, USA), with a sampling time of 1 min. Vials were pre-conditioned for 10 min before microextraction to allow their thermal equilibration.

Volatile compounds were separated on a SLB-5ms capillary column (30 m x 0.25 mm i.d., 0.25 μm film thickness), purchased from Supelco (Bellefonte, PA, USA), with the following operating conditions: initial temperature 40 °C, 4 °C /min up to 180 °C, then 25 °C/min up to 260 °C and a final holding of time of 10 min. Injection was performed in split mode (split ratio 1:200) and temperatures of injection port and transfer line were set at 260 °C. Carrier gas was helium, at a linear flow rate of 36 cm/s. Detector voltage was set at 1.4 kV. Electron impact mass spectra were recorded at 70 eV and the identification of limonene was carried out by comparison of mass spectra and retention times with those of a commercial standard.

Data analysis and modelling

All determinations were expressed as the mean ± standard error (SE) of at least two measurements from two experiment replicates ($n \geq 4$).

β-carotene and limonene degradation data were fitted to a pseudo-first order kinetic reaction and apparent reaction rate constant (k_L, hours^{-1}) at each light intensity (L) were calculated by linear regression:

$$\ln\left(\frac{C}{C_0}\right) = -k_L t \quad (1)$$

165 Where C is β-carotene or limonene content at storage time *t* and C_0 is their content at time zero. Regression significance was evaluated by considering determination coefficients (R^2) and probability value (p).

168 Shelf life equation based on the pseudo first order was as follows:

169 $$SL_L = \frac{\ln a - \ln b}{k_L} \quad (2)$$

170 where SL_L is the shelf life at the selected light intensity (*L*), *a* and *b* are the final (corresponding to the end of shelf life) and the initial quality index value, respectively.

172 **RESULTS AND DISCUSSION**

173 *Microemulsion physical stability upon beverage preparation*

174 Starting from our previous research[3], beverage prototypes were prepared by diluting transparent microemulsion loaded with lemon oil (15% w/w) as flavouring ingredient and β-carotene as bioactive/colorant compound. In these experiments, β-carotene was solubilized in a 3:1 lemon oil-peanut oil mixture before microemulsion preparation. The resulted microemulsions had a mean particle diameter lower than 30 nm, giving reason of yellow transparent systems. This transparency was maintained upon microemulsion dilution to obtain a beverage with pH 2.1. This was demonstrated by the very low turbidity value (~ 0.1 O.D. at 600 nm) of the beverage, confirming data previously reported[3].

182

183 *Light-induced quality depletion of beverage*

184 The beverage delivering β-carotene and lemon oil was stored at 20 °C under different light intensities for increasing time up to 30 days. Transparency, taken as an indicator of emulsion stability, color changes due to β-carotene degradation and flavor profile modifications associated to limonene changes were monitored.

As regard physical stability, no appreciable changes of absorbance at 600 nm were observed under both dark and light conditions. This result, in agreement with Valoppi et al[3], highlighted that microemulsion oil droplets were stable in the diluted systems upon storage, confirming the effectiveness of microemulsions as delivery systems of liposoluble components, such as flavor oils and bioactive compounds.

Given the beverage physical stability during storage, samples were analyzed for β-carotene and limonene content (Figure 1). As the light intensity increased, the bleaching rate of β-carotene also increased, indicating that light exerted a dramatic effect on β-carotene oxidation (Figure 1a). This is in agreement with literature data demonstrating the photosensitivity of β-carotene[8,9]. On the other hand, also limonene content progressively decreased during storage [10,11] (Figure 1b). However, the effect of light appeared different from that observed for β-carotene degradation. The beverages exposed to 800 and 2000 lux showed limonene content changes comparable to that observed in the control sample stored in the dark. Only the exposure to the highest light intensity considered (6000 lux) caused an intense limonene depletion. These observations can be better highlighted by observing the pseudo-first rate constants computed by fitting data shown in Figure 1 as a function of storage time (Table 1). The goodness of the statistical parameters confirmed the exponential decay of both selected indices. Reaction rates showed that storage under increasing light intensity progressively affected β-carotene degradation rate, while a discontinuity point was observed for limonene degradation rate between 2000 and 6000 lux. It could be inferred that due to the high antioxidant capacity of β-carotene, the latter might progressively oxidized, protecting limonene from degradation. However, this protecting effect would not be efficacious when the beverage is exposed to dramatically intense light, such as 6000 lux.

Based on the acquired data, the colour changes associated to β-carotene degradation can be considered the earliest indicator of the quality changes occurring during the beverage storage. Thus, β-carotene kinetic data were used to evaluate the possibility to develop stability, and eventually shelf life, prediction models.

Stability and shelf life prediction models

In order to produce a model to estimate product stability under different lighting conditions, the rate of β-carotene degradation was studied as a function of light intensity (Figure 2). This approach was previously proposed by Manzocco et al. [5,6], dealing with the development of protocols for the shelf life testing of photosensitive foods, such as beverages and oils. These authors proposed the used of light as unconventional acceleration factor in shelf life accelerated test (ASLT) to reduce the time needed to obtain a reliable shelf life estimation of photosensitive foods.

Observing Figure 2, it is evident the linear relation (R^2=0.998) between β-carotene degradation rate and light intensity. This result is consistent with those obtained by Manzocco et al. [5] studying the light dependence of crocin bleaching rate. The linear relation between β-carotene degradation rate and light intensity confirms the exploitability of light as acceleration factor in stability or shelf life tests. Thus, by measuring the bleaching rate under increasing light intensity and then extrapolating the rate at milder conditions, it can be possible to estimate the beverage degradation rate under dark but also under milder light conditions, usually experienced by the product on the retail shelves.

The knowledge of the photo-stability of the beverages could allow to generate a shelf life predicting model based on light as accelerating factor. To convert a stability model into a shelf life model, it is necessary to select the shelf life acceptability limit (that is the *b* value in the equation 2). As reported by Manzocco[12], the choice of acceptability limit value for products without compulsory indications, such as the considered beverages, is prevalently based on company policy. For the considered beverages and based on the equation reported in Figure 2, the shelf life model (eq. 3) was as follows:

$$SL_L = \frac{\ln a - \ln b}{1 \cdot 10^{-5} L + 0.0156} \qquad (3)$$

Equation 3 represents a simple model allowing prediction of the shelf life of the beverage at different light intensities (L). For instance, by choosing the acceptability limit in correspondence of 50% β-carotene degradation and applying equation (3), the shelf life under dark resulted 250 days. On the contrary, considering the typical light intensity of market shelves (about 600 lux), the shelf life became about 86 days.

CONCLUSIONS

The use of transparent microemulsions can be regarded as successful strategy to deliver liposoluble molecules, such as lemon oil and β-carotene, into beverages. The latter resulted physically stable during storage, indicating that no coalescence phenomenon occurred during storage. On the contrary, beverages resulted photosensitive showing intense colour fading and flavour depletion during storage as a consequence of limonene and β-carotene oxidation. This means that in the attempt to develop beverages containing microemulsions as delivery system, the photostability of the delivered compounds should be carefully studied. To this aim, validated mathematical models able to predict shelf life as a function of light intensity are definitively needed to speed up the process.

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289 **Figure captions**

290 Figure 1. β-carotene (a) and limonene (b) changes as a function of beverage storage time 20 °C

291 under different light conditions.

292 Figure 2. Light dependence of first order rate constant (*k*) of β-carotene degradation as a function of

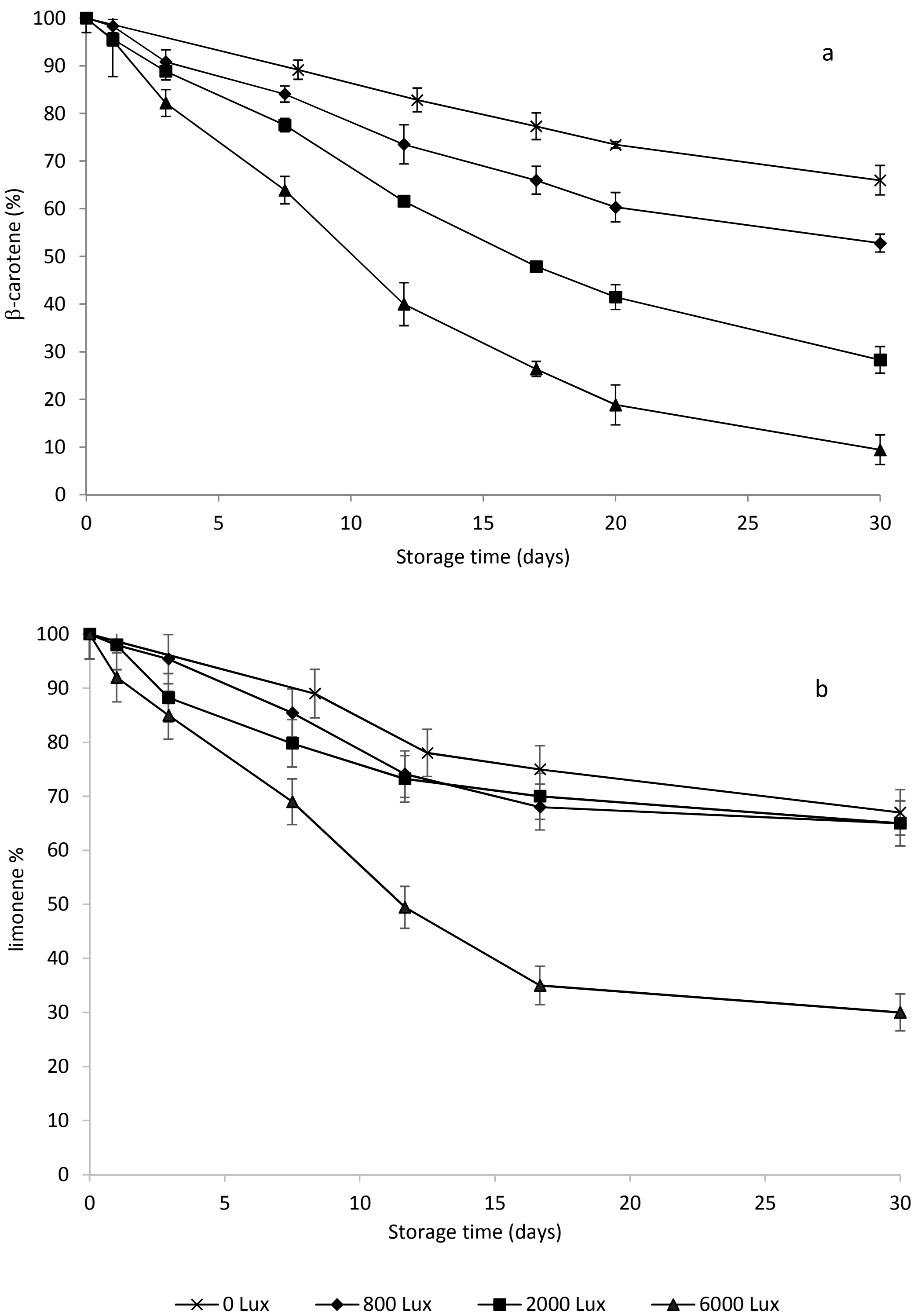
293 light intensity. Linear regression results and determination coefficient are also shown.

294

Table 1. First order degradation rate (k_L) of β-carotene and limonene in beverages stored at 20 °C under different light intensity.

	β-carotene		Limonene	
Light intensity (lux)	k_L (day^{-1})	R^2	k_L (day^{-1})	R^2
0	0.0142±0.0010	0.996	0.0135±0.0021	0.935
800	0.0223±0.0011	0.995	0.0158±0.0026	0.879
2000	0.0430±0.0010	0.984	0.0145±0.0026	0.856
6000	0.0812±0.0023	0.997	0.0432±0.0058	0.915

$P<0.01$



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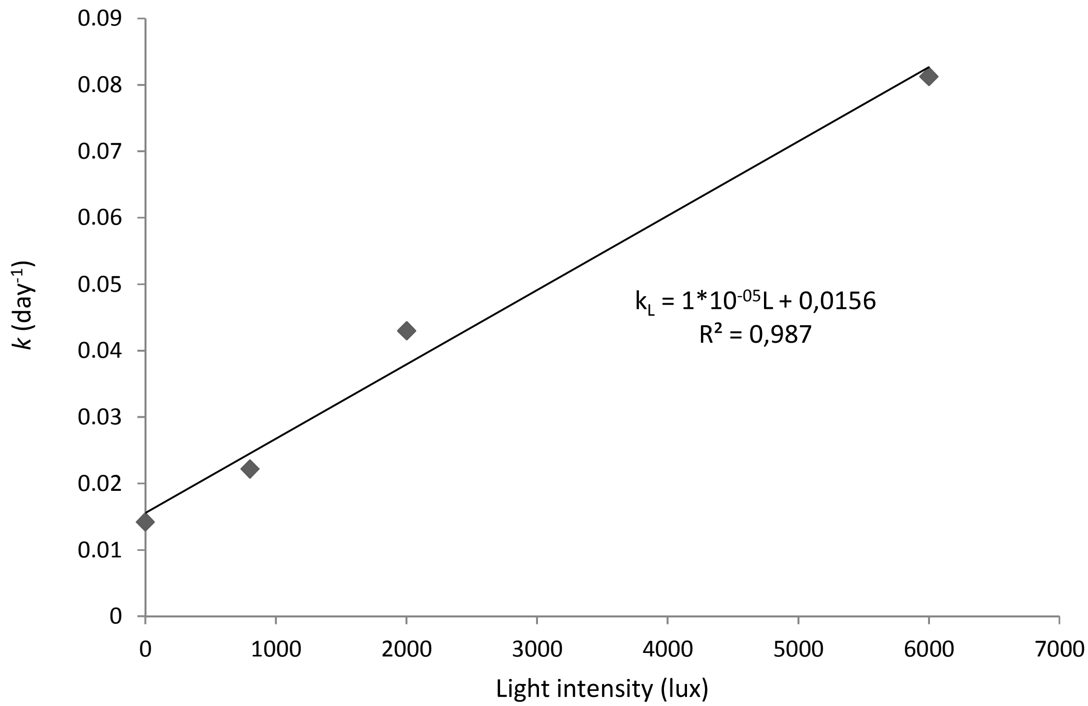

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