Protein Co-habitation: Improving the Photo-Chemical Stability of R-Phycoerythrin in Solid State

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ABSTRACT

The poor photo-chemical stability of R-phycoerythrin (R-PE) has been a bottleneck for its broad-spectrum applications. Inspired by nature, a sustainable strategy of protein co-habitation to enhance the R-PE stability by embedding it in a solid matrix of gelatin, was here studied. Both pure R-PE and fresh phycobiliproteins (PBPs) extracts recovered from Gracilaria gracilis were studied. The incorporation of R-PE in the gelatin-based films (gelatin-RPE and gelatin-PBPs) has improved its photochemical stability for at least 8 months, the longest time period reported so far. These results were evidenced not only from the absorption, but also the emission quantum yield measurements (Φ). Moreover, the photo-stability of gelatin-RPE films upon continuous excitation with AM1.5G solar simulator was tested and found to remain stable up to 23 h after an initial decrease up to 250 min. In the end, another approach was established to allow 100% of photostability for 3 h of exposure to AM1.5G solar simulator by doping the gelatin-based film including phycobiliprotein with n-propyl gallate stabilized with Tween 80, allowing its use as natural-based optically active centers in photovoltaic applications.
R-phycoerythrin (R-PE) is a fluorescent protein found in red algae to surpass the low solar irradiance during the algae growth. Phycobiliproteins in general, and R-PE particularly, are part of a photosynthetic antenna complex which assists in photosynthesis by efficiently capturing and transferring the solar energy to the reaction center where chlorophylls are concentrated (Figure 1a). R-PE comprises a number of billin chromophores (prosthetic groups) stabilized by polypeptide chains ($\alpha$, $\beta$ and $\gamma$) in hexamer ($\alpha\beta\gamma$) units (RCSB.PDB: 1EYX) giving it a bright rose pink appearance. Due to its high molar absorption coefficient ($\sim 1.96 \times 10^6 \text{ M}^{-1}\text{.cm}^{-1}$), thermal stability ($\sim 45 \text{ °C}$) and emission quantum yield of $\geq 80 \%$, R-PE has been used for small scale applications in food as colorant, photodynamic therapy as photosensitizer, flow cytometry and immunoassays. More recently, it has been used in solar cells and innovative liquid-based luminescent solar concentrators as an optically-active center. However, besides the production
limitations, its applications are restrained mainly by the poor photo and chemical stabilities. Like other globular proteins (with small Gibbs free energy of unfolding (ΔG_u; 5-15 kcal.mol⁻¹), R-PE is prone to chemical degradation with time and in aqueous media at room temperature, which also affects its photo emissive properties. To overcome these drawbacks, various studies have been done considering the use of preservatives/osmolytes such as glucose, sucrose, sodium chloride, ascorbic acid, benzoic acid, citric acid, sodium azide and butyl hydroxyl toluene. These have been studied to stabilize R-PE in aqueous formulations, with a maximum of 65 days (between 4 and 35 °C) being reported. Moreover, some other reports have demonstrated that the use of 5 % of NaCl in distilled water represents good results of stability, with only 40 % loss in color after 40 days of incubation in the temperature interval [−5 ; 5] °C. Their mechanism of protection is similar to the one found for conventional protective osmolytes towards globular proteins, which is described by the preferential exclusion of osmolytes from the protein surface; ΔG_u > 0 kcal.mol⁻¹. Moreover, the concept of genetic simplicity of proteins is also applicable to explain the stability of R-PE in salt solutions, which somehow mimics the natural environment of red algae growth, and consequently, the R-PE production. Also, some nanoparticles (Ag⁰) have been reported as efficient crosslinkers to increase the R-PE thermal stability at > 50 °C by preventing the protein aggregation. A commercial aqueous formulation sold by Sigma-Aldrich (150 mM sodium phosphate, 60% ammonium sulfate, 1 mM EDTA, 1 mM sodium azide, pH 7.0), for example, comprises different osmolytes to preserve R-PE for a long period of time at 4-8 °C, but the chemical stability of R-PE at room temperature for a larger period is still a drawback to overcome. Allied to the poor chemical stability of R-PE is its poor photostability promoted by the non-linear optical effects at high irradiance. In this sense, this work will study an efficient approach to improve the chemical and photo stabilities of R-PE allowing thus its use in photonic
The photoluminescent characteristics of R-PE come from 34 chromophores present in its \((\alpha\beta)\gamma\) hexamer unit (5 in each \(\alpha\beta\) unit and 4 in the \(\gamma\) linker polypeptide). These chromophores are further comprised of phycoerythrobilins (PEBs) and phycourobilin (PUB). Both PEBs differ in their inner ring coplanarity, each absorbing at 530 nm (PEB-530) and 560 nm (PEB-560), whereas PUB with different chemical structures absorb at 490 nm (Figure 1b).

**Figure 1.** Schematic of a) singlet excitonic energy transfer between phycobiliproteins and photosystem in the thylakoid membrane, b) absorption spectrum of R-PE showing the absorption peaks corresponding to different chromophores (PEB and PUB) and c) different photo-deactivation channels of R-PE, 1) singlet-singlet excitonic collisions, 2) transition to dark triplets and 3) induced photodegradation by singlet oxygen \(^1\text{O}_2\).
The light harvesting in such absorption bands yields the appearance of an analogous emission spectra formed by a band centered at 575 nm. Moreover, the emission quantum yield is also independent of the excitation wavelength, reinforcing the effective contribution of all chromophores. Therefore, to keep the optical properties, there is the need to ensure their chemical stability in the polypeptide chains. Gaigalas and coworkers\textsuperscript{26} have reported that PUB-490 transfers its absorbed energy to PEB-530 due to strong coupling and, ultimately, to PEB-560 via fluorescence resonance energy transfer, with PEB-560 as the final emissive state. However, upon exposure to AM1.5G solar spectrum or laser beam (514 nm, 30 mW.cm\textsuperscript{-2}), the R-PE aqueous solution becomes non-emissive with consequent losses in the emission quantum yield within a few minutes. Different mechanisms reported so far for include irreversible singlet-singlet excitonic collisions,\textsuperscript{30} the transition to dark triplet state,\textsuperscript{26} and photodegradation by singlet oxygen generated due to the interaction of R-PE triplets with the molecular oxygen as the main causes.\textsuperscript{26,31-35}

The mechanism of photobleaching by singlet oxygen (\textsuperscript{1}O\textsubscript{2}) to molecular oxygen (\textsuperscript{3}O\textsubscript{2}) saturated solution of PBPs was already experimentally proved.\textsuperscript{33-34} Among these, Zhang et al.\textsuperscript{33} showed that R-PE has the least tendency to generate \textsuperscript{1}O\textsubscript{2} compared to other phycobiliproteins, being thus less susceptible to photobleaching by \textsuperscript{1}O\textsubscript{2}. Also, Tapia et al.\textsuperscript{34} showed that \textsuperscript{1}O\textsubscript{2} has the least contribution towards photobleaching of prosthetic groups (chromophores) compared to other deactivation channels like singlet-singlet excitonic collisions and transition to dark triplet state. These observations also find support from the poor photostability results reported in aqueous solution of PBPs in the presence of antioxidants like ascorbic acid, n-propyl gallate, 1,4-diazabicyclo [2,2,2] octane, dithiothreitol, and sodium azide to neutralize \textsuperscript{1}O\textsubscript{2},\textsuperscript{26,32-34} thus indicating that singlet oxygen is a minor culprit. Zheng et al.\textsuperscript{30} proposed another deactivation channel of singlet-singlet excitonic annihilation at photon densities in a range of \textbf{8×10^{14} – 1×10^{17}} photons.cm\textsuperscript{-2}. However, at high
excitation intensity, the transition of singlet to dark triplets has been considered the key deactivation channel, indirectly facilitating the photobleaching by $^{1}\text{O}_2$ in an $^{3}\text{O}_2$ saturated environment. From single molecule recrossing experiments, Burrows et al.\textsuperscript{26} showed that energy transfer to the tandem dye (AlexaFluor-647) conjugated to R-PE, significantly improved its photostability and light tolerance by avoiding the formation of the triplet state, thus further ascertaining transition to dark triplet state as the major culprit of photobleaching. However, the existence of the triplet state of R-PE is reported only at 77 K,\textsuperscript{36} with no direct experimental evidence at room temperature. Stadnichuk et al.\textsuperscript{36} reported the R-PE phosphorescence at 720 and 820 nm with triplet lifetimes of 2.5 ms and 6 ms at 77K.

While most works on the PBPs’ photo-chemical stability are reported for aqueous systems, its long-term applications in food and photonic devices also require them stable in solid state, which is also a condition of utmost relevance. Examples include the use of silica by the sol-gel method,\textsuperscript{37} polysaccharide hydrogels\textsuperscript{38} and metal organic frameworks (MOF).\textsuperscript{39} From relative absorption measurements, Chen et al.\textsuperscript{37} reported that phycoerythrin is 60% more photostable in tetramethoxy-silane gel upon continuous irradiation with 514 nm laser (30 mW cm$^{-2}$) than in phosphate buffer for 250 min. Mulder et al.\textsuperscript{38} self-assembled phycoerythrin, phycocyanin and allophycocyanin in a polyacrylamide hydrogel for application in luminescent solar concentrators. The assembled system showed smooth singlet energy transfer via fluorescence resonance energy transfer with an optical quantum efficiency (fraction of incident photons that is emitted from the edges of the luminescent solar concentrator) of 12.5%.\textsuperscript{38} However, the emission properties were lost upon drying the hydrogel complex, therefore facing durability issues. Recently, solid-confinement of R-PE in a blue MOF was reported\textsuperscript{39}. Interestingly, the authors claimed that R-PE denatured inside the MOF, which blocked PUB to PEB energy transfer. The denatured R-PE inside the MOF showed dual
color fluorescence at 518 nm (green) and at 600 and 647 nm (red). In this case, the film showed efficient white light emission upon excitation at 405 nm. Upon continuous illumination with 405 nm LED for 24 hours, the emission intensities at 518 nm, 600 nm and 647 nm remained at 84.8%, 84.9% and 85.5%, thus indicating its high photostability.

In nature, the PBPs appear as multimeric complexes, anchored on the cell membrane in a crowded vitreous environment (Figure 1a). Therefore, we think that trapping R-PE in a vitreous environment could turn out to be a better strategy to photo and chemically stabilize it. Herein, a gelatin matrix was applied as a simple and innovative strategy of protein co-habitation for long term photochemical stability of R-PE. This strategy is inspired by the biochemical cooperativity of structural and functional proteins in vivo wherein they function together without changing each other’s chemical structure.\textsuperscript{40-41} Since a gelatin hydrogel has recently been reported to protect the triplet state from quenching by molecular oxygen,\textsuperscript{42} the gelatin-RPE films are expected to directly exclude the $^1$O$_2$ induced photodegradation channel due to the unavailability of molecular oxygen. In this work, and to have a clearer idea of the mechanism, two R-PE samples were investigated, a commercial one from Sigma-Aldrich and an extract rich in PBPs (Figure 2a) produced in-house after extracting it from the red algae \textit{Gracilaria gracilis}. The detailed procedure applied to produce the extracts rich in PBPs is given in the Experimental Section and Scheme S1 from ESI. The sodium dodecyl sulfate protein gel electrophoresis (SDS-PAGE) of the extract showed bands corresponding to $\alpha$, $\beta$, $\gamma_1$ and $\gamma_2$ subunits of R-PE at 18.2 kDa, 20.6 kDa, 31.6 kDa and 34.6 kDa, respectively (Figure 2b). The absorption spectrum of PBPs extract (Figure 2c) showed peaks corresponding to different chromophores of R-PE (PUB-499, PEB-542 and PEB-565), phycocyanin at 620 nm and a small hump for allophycocyanin at 650 nm. Those peaks are red-shifted in the emission spectra to 581 nm (R-PE), 645 nm (phycocyanin) and 664 nm
(allophycocyanin), respectively (Figure S1b, ESI). The circular dichroism spectra of PBPs in solution (Figure 2d and Figure S2 from ESI) showed bands corresponding to all-α secondary structure with a positive band at 194 nm and negative bands at 208 and 221 nm, which agrees with the circular dichroism spectrum of pure R-PE from Sigma-Aldrich (Figure 2g and Figure S3 from ESI). The lower ellipticity of the band at 222 nm compared to the one at 208 nm for R-PE can be attributed to some impurities of phycocyanin and allophycocyanin in PBPs extract, as indicated by the absorption spectrum. Compared to PBPs extract, the pinkish colored aqueous solution of pure R-PE (Figure 2e) showed prominent absorption peaks for PUB-498, PEB-540 and PEB-566 (Figure 2f) and all-α secondary structure (Figure 2g). Contrary to PBPs extract, the pure R-PE showed emission peaks at 581 nm along with a small hump at 625 nm (Figure S1b from ESI). The fluorescence decay curves of PBPs and R-PE aqueous solutions reveal a single exponential decay, yielding to fluorescence lifetimes values of 3.41±0.02 ns and 3.65±0.05 ns, respectively (Figure S4 from ESI).

Figure 2. a) Photograph of the extract rich in PBPs from Gracilaria gracilis b) SDS-PAGE of PBPs extract showing bands (kDa) of the R-PE subunits. c) Absorption spectrum of PBPs extract showing bands (kDa) of the R-PE subunits. d) Absorption spectrum of PBPs extract showing bands (kDa) of the R-PE subunits. e) Absorption spectrum of PBPs extract showing bands (kDa) of the R-PE subunits. f) Absorption spectrum of PBPs extract showing bands (kDa) of the R-PE subunits. g) Absorption spectrum of PBPs extract showing bands (kDa) of the R-PE subunits.
showing bands corresponding to chromophores of R-PE (PUB and PEB), phycocyanin and allophycocyanin. **d)** Far-UV circular dichroism spectrum of PBP showing typical secondary structure corresponding to all-α type. **e)** Photograph of pure R-PE solution. **f)** Absorption spectrum of PBPs extract showing bands corresponding to chromophores of R-PE (PUB and PEB) and **g)** Far-UV circular dichroism spectrum of PBPs extract showing typical secondary structure corresponding to all-α type.

The films were prepared by dissolving each sample (commercial R-PE or extract of PBPs) in hot gelatin sol at 45 °C, followed by casting on a glass or plastic plates (see the Experimental Section of ESI). Optically transparent films obtained after air-drying on a glass plate are shown in **Figures 3a** and **3b**. Upon translating from aqueous solution to the solid film, the PBPs showed a decrease in absorbance by 6.5-fold whereas, the commercial R-PE showed a 2.5-fold decrease (**Figures S5b** and **S5e** from ESI), hence indicating the suppression of effective concentration of the proteins due to aggregation. The smaller red shift (~1.0 nm) observed in the absorption spectra for gelatin-PBP and gelatin-RPE films, when compared with that found in water supports this observation (**Figures S5c** and **S5f** from ESI).

The emission spectrum of the gelatin-PBP film shows a band peaking at 578 nm and a high-wavelength range shoulder (650 nm), similarly to that found for the gelatin-RPE film, despite a deviation of the shoulder top longer wavelength (630 nm). The emission peaks of R-PE (at 578 nm) in both samples are blue shifted (~2 nm) compared to that found in the aqueous solution (**Figure S1b** from ESI), which indicates aggregation of chromophores. Billiproteins are well documented to undergo intensity and wavelength change upon variations in the surrounding environment due to solvatochromism.⁴³ Although the peaks corresponding to phycocyanin and
allophycocyanin in PBPs are severely suppressed in the films, the fluorescence resonance energy transfer between R-PE and phycocyanin is still operative as observed from the excitation spectrum (Figure S6 from ESI). The fluorescence decay profiles of both films showed in Figure 3b and 3c, are well described by single exponential decay (Figure 3d and 3e). Interestingly, the lifetime value in both films increased to 7.27±0.01 ns (gelatin-PBP) and 7.56±0.07 (gelatin-RPE) compared to that in aqueous solutions (Figure S4 from ESI). This may be probably explained by the dispersion of the molecules in the film reducing the molecule-molecule interactions and concomitantly increasing the lifetime.44

The impact of having films was also quantified by the determination of the emission quantum yield (Φ). The Φ value measured for the aqueous solution of PBPs extract was 39±4 %, decreasing to 17±2 % upon translating to gelatin-PBP film, possibly due to aggregation yielding fluorescence quenching.43 A similar behavior was observed for pure R-PE, whose Φ values decreased from 64±6 % to 46±5 % upon translating to gelatin-RPE film.
Figure. 3. Photographs of a) gelatin(15%)-PBP films, b) gelatin(15%)-RPE film. c) Emission spectra of gelatin-PBP (black curve) and gelatin-RPE (red curve) films excited at 498 nm. d, e) Emission decay curves excited at 330 nm and monitored at 578 nm. The solid lines represent the data best fit ($R^2 > 0.98$), using a single-exponential function $I(t) = I_1 e^{-(t-t_0)/\tau_1}$ ($t_0 = 23$ ns, related with the excitation prompt). The respective residual plot is shown on f).

After, the photo-chemical stability dependence with time was studied by comparing the performance of gelatin-PBP and gelatin-RPE films during 8 months using the parameters absorption and $\Phi$ (Figures 4a, 4b, Figure S7 from ESI). The absorbance of the gelatin-PBP film remains nearly constant after 274 days of incubation at room temperature, showing the significant chemical stability of R-PE when entrapped in the gelatin film. Moreover, the gelatin-RPE film revealed chemical stability in the initial 84 days followed by $\sim$23% loss in absorbance after 253 days. The time dependency of photo-chemical stability (room temperature) was measured for 8
months using $\Phi$ (Figure 4b, Table 1). Interestingly, both gelatin-PBP and gelatin-RPE films showed no change in $\Phi$, indicating photo-chemical stability at room temperature. This behavior was further observed for one month of experiment when increasing the gelatin concentration from 15 % to 40 % (Figure S8, Table 1).

Figure 4. a) Time dependence of the absorbance at 565 nm of gelatin-RPE and gelatin-PBPs films for 253 and 274 days at room temperature. b) Comparative emission-quantum yield ($\Phi$) of gelatin-RPE (15%) and gelatin(15%)-PBPs films after incubation for 8 months. c) Combined absorption and emission spectra of gelatin(15 %)-PBPs film. d) Time dependent $\Phi$ of gelatin(40%)-RPE and gelatin(40%)-PBPs films upon continuous exposure to AM1.5 solar simulator for 1380 min.
Table 1. Time dependency of the emission quantum yield of pure gelatin-RPE and gelatin-PBPs films at room temperature from 1 to 8 months ($\lambda_{ex}$=498 nm).

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\Phi / %$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Month 0</td>
</tr>
<tr>
<td>Gelatin(15%)-RPE film</td>
<td>47.0 %</td>
</tr>
<tr>
<td>Gelatin (15%)-PBPs film</td>
<td>17.0 %</td>
</tr>
<tr>
<td>Gelatin(40%)-RPE film</td>
<td>31.7 %</td>
</tr>
<tr>
<td>Gelatin (40%)-PBPs film</td>
<td>15.0 %</td>
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Indeed, being colloidal particles, proteins undergo frequent collisions due to molecular diffusion via Brownian motion in aqueous solution. Collisions cause the protein aggregation, due to exposed hydrophobic surfaces, leading to degradation with time. Transformation to solid state restricts their diffusion and hence obdurate the chemical degradation. The photo-chemical stability of R-PE in the gelatin matrix may have a significant impact if using it as colorant in food applications. Furthermore and, since gelatin is an edible protein, the gelatin-RPE formulations can be used as long term stable colorants in food products. The photo-chemical stability of gelatin-RPE can be accounted to protein-protein interactions between R-PE and gelatin, thus providing a suitable environment for R-PE, mimicking the environment found in its natural environment in algae. These protein-protein interactions were evidenced from their combined circular dichroism spectrum in solution at low concentration showing bands corresponding to both the proteins (see
Figure S9 from ESI). Cooperative interactions in solution remain the same in solid state, which was evidenced by the retention of all photophysical characteristics of R-PE and no phase separation observed in the film. Further evidences demonstrating the importance of protein-protein interactions were obtained by testing the polysaccharide sodium alginate (NaAlginate) as a matrix for PBPs, which phase separated from PBPs upon drying to solid film (see Figure S10a) and also by the blunted absorption spectrum peaks of R-PE in NaAlginate-PBPs films (see Figure S10b from ESI).

Due to its natural abundance and large spectral coverage (absorption/emission) ranging from 400-750 nm, PBPs represent a suitable case of sustainable artificial light harvesters in photovoltaics.\textsuperscript{16-17,28-29} PBPs can be counted as type A luminophores, with Stokes-shift of 12-15 nm from the absorption maximum of R-PE (565 nm), thus reducing the reabsorption (Figure 4c), generally encountered with the synthetic dyes.\textsuperscript{48} However, as previously discussed, its poor photostability upon continuous excitation is a major bottleneck.\textsuperscript{26-27} This phenomena was also studied in this work by checking the films photostability when exposed to continuous excitation with AM1.5 solar simulator, by determining the $\Phi$ for 23 hours considering the films with 20 % to 40 % of gelatin to keep away the molecular oxygen (Figure S11 from ESI and Fig. 4d).\textsuperscript{42,49} To maximize the photostability, films doped with singlet oxygen quenchers like n-propyl gallate and ascorbic acid commonly used as stabilizers, were investigated (Figure S11 from ESI). Compared to the aqueous environment, where $\Phi$ decreased from 64 % to 2 % after 75 min of continuous exposure to solar simulator (AM1.5G), the gelatin-RPE and gelatin-PBPs films showed, respectively, a decrease of 30 % to 16% and 13 % to 9% after 180 min, and a decrease of 30 % to 8 % and 13 % to 6 % after for 360 min (Figure S11 and Table S1 from ESI). Meanwhile, the addition of n-propyl gallate-Tween 80 as dopant to gelatin(20%)-RPE film retained 100% of photostability up
to 180 min, which is the longest period reported up to now. However, this was followed by a drastic decrease of $\Phi$ from 32 % to 9 % after 540 min, which was maintained constant up to the 23 hours of exposure. The addition of ascorbic acid quenched the fluorescence of R-PE to $\Phi= 6.0 \pm 0.6 \%$ and remained constant after exposure up to 23 hours (Figure S11 and Table S1 from ESI).

To infer about the effect of the doping agent, the concentration of gelatin was increased to 40 % in the film and exposed to solar simulator. Upon increasing the gelatin concentration to 40 %, the fluorescence lifetime values in both films decreased to $\tau_1 = 6.12 \pm 0.08$ ns (gelatin-RPE) and $\tau_1 = 6.45 \pm 0.11$ ns (gelatin-PBP), which may be explained by the increased films rigidity Figure S12, from ESI). The $\Phi$ values became constant ($\Phi = 8.0 \pm 0.3 \%$) for gelatin(40%)-R-PE film and $\Phi = 6.5 \pm 0.2 \%$ for the gelatin-PBPs film after an initial decrease found up to 4 hours of exposure (Figure 4d, Table S2 and Figure S13 from ESI). It is highly likely that the R-PE fluorophores present at the surface of the film undergo a transition to non-emissive triplet state according to route 2 described in Figure 1. In the absence of molecular oxygen in the film, the chances of photodegradation by singlet oxygen generated by route 3 of Figure 1 are unlikely.

**Figure 5.** Schematic of the enhancement of photochemical stability of R-PE upon protein co-habitation with gelatin in solid state. R-PE (RCSB.PDB: IEYX) and gelatin (RCSB.PDB: 1V7H).
From these results, it seems that R-PE at the core of the film remains photo-physically active, whereas the one at the interface undergoes photobleaching. Moreover, the inactivated fluorophores at the interface may be acting as sacrificial protectants along with gelatin, an argument further supported by the interfacial passivation obtained by using the surfactant Tween-80, for which the film retained 100 % of its photostability for 180 min. The obtained photostability of R-PE in the gelatin film is much higher than the one in water, being the best reported up to now (Figure 5).\textsuperscript{37}

Finally, the stability of R-PE in the films was also checked against the temperature increase denoted in the film upon continuous irradiation by the solar simulator. In this sense, the temperature was monitored along all the experiments, revealing a maximum increase of 4°C (\textasciitilde23 °C to 27 °C), which is within the limits of thermal stability of R-PE (\textasciitilde45 °C), thus eliminating potential negative effects on the R-PE stability.

In conclusion, this work proposes a sustainable strategy to overcome the limitations of photochemical stability of R-PE. By applying the rational of protein co-habitation of R-PE with gelatin in the solid state, we have developed a material in which R-PE remains photo-chemically stable up to 8 months at ambient conditions. Moreover, the co-habitation of R-PE with gelatin was also improved upon doping it with Tween 80-n-propyl gallate, which has resulted in the retention of 100 % of initial quantum yields of the fluorescent protein for 180 min, occurring this to be the maximum period reported so far, an excellent and promising result to increase the potential of application of R-PE in food sector.

\textbf{ASSOCIATED CONTENT}

\textbf{Supporting Information.} Experimental Section includes the sections Materials, Methods, Extraction methodologies to recover PBPs from \textit{Gracilaria gracilis} and prepare the PBPs extract,
processes to prepare the gelatin-RPE and gelatin-PBPs films (Scheme S1 and Figures S1 to S13) are provided in ESI.

AUTHOR INFORMATION

Notes

The authors declare no competing financial interests.

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