LWT - Food Science and Technology 138 (2021) 110776

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Vegetable oils protect phycocyanin from thermal degradation during cooking of spirulina-based "crostini"

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ARTICLE INFO

Keywords:
Leavened bakery foods
Spirulina
Phycocyanin
Extra virgin olive oil
Sunflower oil

ABSTRACT

Phycocyanin is a well-known bioactive pigment contained in cyanobacteria, such as *Arthrospira platensis* (commonly known as spirulina), and used in the food and beverage industry. One of the main problems that affects spirulina-based bakery products is phycocyanin degradation during cooking, being this pigment extremely sensitive to heat.

The main goal of this work was to evaluate the protective effect against phycocyanin degradation of extra virgin olive (EVO) oil or sunflower oil, by applying thermal treatments directly on *A. platensis* F&M-C256 biomass and on *A. platensis* F&M-C256-based "crostini", as well as on phycocyanin powder. After cooking, *A. platensis* F&M-C256 "crostini" incorporated with EVO or sunflower oil (10g oil/100g of dough) maintained about 90% of the phycocyanin originally present in the cyanobacterial biomass. When pure tocopherol was added to the biomass in the same amounts, a significant protective effect against phycocyanin degradation was observed. Tocopherol contained in EVO and sunflower oils is the putative main responsible for the protective action against phycocyanin degradation. Therefore, the incorporation of vegetable oils into the dough can be a useful tool for the food industries that use *A. platensis* biomass and/or phycocyanin as a natural food coloring and bioactive component for bakery products.

1. Introduction

"Crostini" is a typical Italian leavened food product, obtained by cooking and successive toasting to reach a humidity <10% on final product weight (DPR, 1993). New bakery products with a high nutritional value and an innovative taste, consumable on a daily basis, are increasingly requested. To enhance the nutritional quality of bakery products, the addition of functional ingredients has been considered (e.g., wheat germ, fibers, microalgae) (Batista et al., 2017; Graça, Fradinho, Sousa, & Raymundo, 2018; Ragaee, Guzar, Dhull, & Seetharaman, 2011; Rizzello, Cassone, Coda, & Gobbetti, 2011).

The cyanobacterium *Arthrospira platensis*, commercially known as "spirulina", is considered a "safe food source" and authorized as food in several countries. Moreover, the safety of *A. platensis* for human consumption is supported by its long history of use as food ingredient (Abdulqader, Barsanti, & Tredici, 2000). *A. platensis* has been proposed as novel ingredient for the production of functional beverages (Niccolai, Shannon, et al., 2019) or foods such as fish burgers (Barkallah et al., 2019), feta cheese (Mazinani, Fadaei, & Khosravi-Darani, 2016), cookies (Batista et al., 2017), gluten-free pasta (Fradinho et al., 2020), crackers (Batista et al., 2019), and "crostini" (Niccolai, Venturi, et al., 2019). *A. platensis* is considered a source of digestible proteins (up to 70g/100g of dry weight) (Niccolai, Chini Zittelli, Rodolfi, Biondi, & Tredici, 2019), iron, γ-linolenic acid, sulfated polysaccharides and phycocyanin (Gutiérrez-Salmeán, Fabila-Castillo, & Chamorro-Cevallos, 2015; Kulshreshtha, Jarouliya, Bhadauriya, Prasad, & Bisen, 2008). Phycocyanin (PC) is a well-known bioactive substance contained in *A. platensis* (generally in the range of 8–12g/100g of cell dry weight) as well as in other cyanobacteria and red algae (Li, Gao, Zhang, & Chu, 2006; Yan et al., 2011). PC from *A. platensis* is sold as natural coloring agent and it is also used in the food and beverage industry to color ice-creams, chewing gums, candies and other sweet delicacies (Kuddus, Singh, Thomas, & Al-Hazimi., 2013; Martelli, Folli, Visai, Daglia, & Ferrari, 2014). PC is considered a functional blue dye since it is known to have

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https://doi.org/10.1016/j.lwt.2020.110776
Received 26 June 2020; Received in revised form 7 November 2020; Accepted 13 December 2020
Available online 15 December 2020

many biological activities such as antioxidant, immune-modulatory, neuroprotective and anticancer activities (Lee et al., 2013; Romay, Gonzalez, Ledon, Remirez, & Rimbau, 2003).

PC is known to be extremely sensitive to heat and pH (Abalde, Betancourt, Torres, Cid, & Barwell, 1998; Chaiklahan, Chirasuwan, & Bunnag, 2012; Martelli et al., 2014). This sensitivity increases its production cost and decreases its biological functions during production and storage (Choi & Lee, 2018; Sarada, Pillai, & Ravishankar, 1999). One of the principal problems that affect PC-based bakery products is the degradation of the pigment during cooking, including denaturation of the proteins and loss of bioactive functions. Several authors, in order to confer greater stability during storage, encapsulated phycocyanin in synthetic or biological polymers generally by extrusion techniques or electrospraying (Schmatz, da Silveira Mastrantonio, Costa, & de Morais, 2020; Yan, Liu, Jiao, & Qin, 2014). Most of the published papers consider the use of technologies and preservatives that are not appropriate for food application such as silica matrix (Li, Yang, & Cao, 2009), sodium azide, and dithiothreitol (Mishra, Shrivastav, & Mishra, 2008), or ingredients that are not suitable for all bakery products, e.g., sugars and polyhydric alcohols (Petersen, Jonson, Fojan, Wimmer, & Pedersen, 2004; Earthrise® Nutritional, 2019). Moreover, the above-mentioned works focused on mild thermal treatments, with temperatures lower than 80 °C. The identification of a protective agent against PC degradation for cooking temperatures higher than 100 °C is of particular interest, especially for bakery products. In a previous work, Batista et al. (2017) used two concentrations, 2g/100g and 6g/100g, of *Arthrospira platensis* F&M-C256 for the production of cookies. After baking at 110 °C for 40 min, the cookies presented only 7.4 and 10.8% of the phycocyanin present in the microalgal biomass, for 2 and 6g/100g, respectively. Niccolai, Venturi, et al. (2019) used *A. platensis* F&M-C256 biomass in "crostini" production at three different concentrations, 2, 6, and 10g/100g. In spite of the baking at 160 °C for 11 min and successive toasting at 140 °C for 11 min, more than 95% of phycocyanin was still present. Taking into account that the main difference between "crostini" and cookies recipes was the lipid matrix incorporated into the dough, extra virgin olive oil in "crostini" and reduced-fat margarine in cookies, we hypothesized a protective effect due to different tocopherol amounts occurring in the two lipid matrices. Tocopherol (commonly called vitamin E) has shown protective effects on proteins when integrated into foods such as pork patties and cooked pork meat (Haak, Raes, & De Smet, 2009; Salminen, Estévez, Kivikari, & Heinonen, 2006). Not only extra virgin olive oil but also other vegetable oils, e.g., sunflower oil, are rich sources of α-tocopherol. Sunflower oil contains about 40 mg α-tocopherol/100g oil and extra virgin olive oil generally contains 14–18 up to a maximum of 37 mg α-tocopherol/100g oil (USDA, Food Composition Database; Psomiadou, Tsimidou, & Boskou, 2000). To the best of our knowledge, no studies in the literature report the tocopherol protection effect on phycocyanin during thermal treatment. Therefore, the aim of this work was to evaluate the protective effect against PC degradation by two vegetable oils and the role of tocopherol. The effect was evaluated after thermal treatment directly on *A. platensis* F&M-C256 biomass as well as on phycocyanin powder and successively during the manufacture of *A. platensis*-based "crostini".

2. Materials and methods

2.1. A. platensis F&M-C256 biomass production and phycocyanin origin

Biomass of *Arthrospira platensis* F&M-C256, a strain belonging to the Culture Collection of Fotosintetica & Microbiologica S.r.l. (Florence, Italy), was produced at Azienda Agricola Serenissima S.S. (Conche di Codevigo, Padova, Italy). The cyanobacterium was cultivated in Zarrouk medium (Zarrouk, 1966) in GWP®-II photobioreactors (Tredici, Rodolfi, Biondi, Bassi, & Sampietro, 2016) in semi-batch mode, harvested by filtration, and the biomass was washed with tap water to remove excess bicarbonate. The biomass was then dried at low temperature (33 °C) for 20 h and the obtained flakes were stored at −20 °C until use. Proteins, carbohydrates, lipids, moisture, ash, and phycocyanin of the algal powder were 67.3 ± 0.01, 12.5 ± 0.02, 6.7 ± 0.002, 7.7 ± 0.1, 5.8 ± 0.1, and 8.1 ± 0.1g/100g, respectively.

Phycocyanin powder, with the purity of 1.5 (OD 620/280), commercialized as "Blue spirulina phycocyanin powder", was purchased from Xi'an Nate Biological Technology Co. (Shaanxi, China). The product specifications from the certificate of analysis issued by Xi'an Natural Field Bio-Technique Co. (Xi'an, China) are reported in Supplementary Table 3.

2.2. A. platensis F&M-C256 biomass and pure phycocyanin thermal treatments

To evaluate the possible protective action of extra virgin olive (EVO) oil against phycocyanin degradation during cooking, biomasses of *A. platensis* F&M-C256 (1g) and phycocyanin powder (1g) were mixed with 0.1g EVO oil and heated at 50, 100 and 160 °C for 10, 20 and 40 min in a static oven (DRY-Line, VWR International, Milan, Italy). *A. platensis* F&M-C256 biomass and phycocyanin powder without EVO oil incorporation (controls) were also heat-treated. The treated samples were stored at −18 °C within hermetic containers, protected from light, for phycocyanin quantification.

In order to evaluate the contribution deriving from tocopherol contained in the EVO oil in protecting phycocyanin, a trial in which *A. platensis* F&M-C256 biomass was mixed with pure α-tocopherol (Sigma-Aldrich, St. Louis, MO, USA) was also carried out. To be well-dissolved, α-tocopherol requires lipid matrixes. Before thermal treatment, pure α-tocopherol was dissolved in melted butter at different concentration. Then, these blends were mixed with *A. platensis* F&M-C256 biomass (1g) in order to obtain three final concentrations: 0.5 mg α-tocopherol/100g of biomass (the lowest concentration), 1.5 mg α-tocopherol/100g of biomass, and 2.5 mg α-tocopherol/100g of biomass (the highest concentration). The samples were then heated at 160 °C for 10, 20 and 40 min in the static oven. *A. platensis* F&M-C256 biomass without α-tocopherol inclusion (control) was also heat-treated. The obtained samples were stored as previously described.

2.3. "Crostini" preparation

Sourdough was prepared and treated as reported by Niccolai, Venturi, et al. (2019). The recipe of *A. platensis* F&M-C256 "crostini" is reported in Table 1.

A. platensis biomass was added at 6g/100g level by replacing total flour. Doughs were prepared by adding butter (Coop Italia, Bologna, Italy), EVO oil (Coop Italia, Bologna, Italy) or sunflower oil (Coop Italia, Bologna, Italy) as lipid matrix. As indicated on the label, butter, EVO oil, and sunflower oil contained 0.0024g/100g, 0.016g/100g and 0.042g/100g of tocopherol, respectively. A control "crostini" (C) without the incorporation of a lipid matrix was also prepared (Table 1). The ingredients were mixed in a twin arm mixer (Bernardi S.r.l., Cuneo, Italy)

Table 1
"Crostini" recipes (g/100g).

Ingredients	Control "crostini"	Butter "crostini"	EVO oil "crostini"	Sunflower oil "crostini"
Sourdough	25	25	25	25
Wheat flour	41	41	41	41
Water	27	14.5	17	17
Butter	0	12.5	0	0
Extra virgin olive oil	0	0	10	0
Sunflower oil	0	0	0	10
Salt	1	1	1	1
A. platensis F&M-C256	6	6	6	6

at room temperature for 10 min at 50 rpm mixing rate. After 25 min of proofing at 25 °C, the doughs were divided into batches of 300g, to obtain molds of 25 cm length and 1.8 cm diameter. Afterward, the molded doughs were fermented at 30 °C for 2 h and baked at 160 °C for 11 min "Crostini" were obtained by cutting the baked molds in pieces of 1.1 cm thickness and toasted at 140 °C for 11 min. After cooling, they were powdered for phycocyanin quantification and stored in a freezer within hermetic containers, protected from light.

2.4. Phycocyanin determination

Phycocyanin content in *A. platensis* F&M-C256 biomass, in phycocyanin powder, and in "crostini" was determined according to Herrera, Boussiba, Napoleone, and Hohlberg (1989). This method is based on the extraction with 1g calcium chloride in 100 mL deionised water at pH 6.8 (20 °C) and consequent spectrophotometric quantification at 620 nm and 280 nm using a UV–Vis spectrophotometric reader (Cary 60 UV–Vis, Agilent Technologies, La Jolla, CA, USA).

2.5. Statistical analysis

Statistical analysis of the experimental data was performed using STATISTICA from StatSoft (version 8.0), Statgraphics Centurion XV from StatPoint Technologies Inc., and GraphPAD Prism, through variance analysis (one-way ANOVA and three-way ANOVA) (significance level of 95%, $p < 0.05$), by the Scheffé test – *Post Hoc* Comparison (significance level of 95%, $p < 0.05$) and through ANOVA followed by Multiple Range Tests to determine the Least Significant Differences (LSD) (significance level of 95%, $p < 0.05$). All analyses were conducted at least in triplicate and presented as average ± standard deviation.

3. Results and discussion

3.1. Effect of extra virgin olive oil against phycocyanin degradation in heat-treated A. platensis F&M-C256 biomass

To verify the effect of EVO oil against phycocyanin degradation, *A. platensis* F&M-C256 biomass, incorporated or not with EVO oil, was heat-treated. Three temperatures and three times were selected: 50 °C (a temperature that does not affect phycocyanin degradation) (Chaiklahan et al., 2012), 100 °C (an intermediate temperature), and 160 °C (the temperature adopted for "crostini" cooking) for 10, 20, and 40 min. Three-way ANOVA was carried out in order to investigate the effect of the three considered parameters. The phycocyanin contents (g/100g) of heat-treated *A. platensis* biomass incorporated or not with EVO oil are presented in Fig. 1. The results of the three-way ANOVA pointed out that only the interaction temperature-time was statistically significant ($p < 0.0001$) in determining the residual content of phycocyanin in *A. platensis* F&M-C256 biomass after thermal treatment (Supplementary Table 1). Therefore, at the increase of temperature, a greater effect of time occurred. As the temperature of thermal treatments and exposure time increases, a higher degradation of phycocyanin in the biomass is observed (Fig. 1), as also reported by several authors (Antelo, Costa, & Kalil, 2008; Böcker et al., 2019; Chaiklahan et al., 2012; Choi & Lee, 2018).

Drops of phycocyanin extracted from heat-treated *A. platensis* F&M-C256 biomass not incorporated with EVO oil are shown in Fig. 2. A lower intensity of blue coloring of the phycocyanin drops extracted from the biomass as temperature and treatment time increase was observed.

Phycocyanin content was also strongly affected ($p < 0.0001$) by EVO oil. In most of the samples, after each thermal treatment, *A. platensis* F&M-C256 biomass incorporated with EVO oil presented a higher phycocyanin content compared to the biomass without EVO oil (Fig. 1). It is interesting to note that at high baking temperatures, a greater protective effect from oil occurred compared to lower temperatures, with consequent higher phycocyanin content in the biomasses

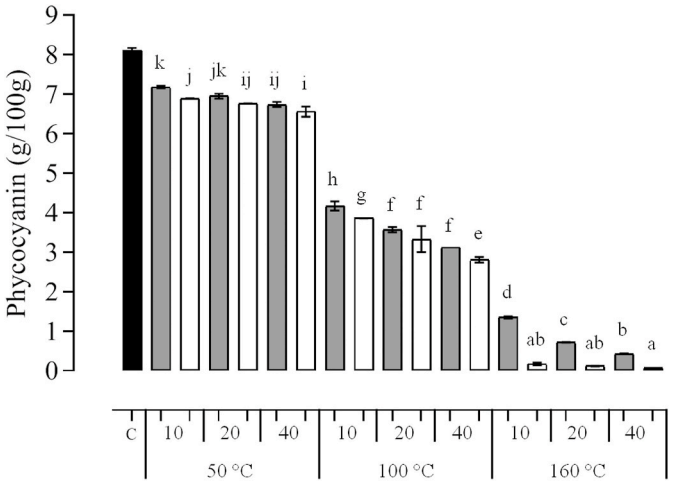

Fig. 1. Phycocyanin content (g/100g) of *A. platensis* F&M-C256 biomasses incorporated with EVO oil (grey columns) and of *A. platensis* F&M-C256 without EVO oil (white columns) after thermal treatment at 50, 100, and 160 °C for 10, 20 or 40 min. Results are expressed as average ± standard deviation (n = 3). Different letters correspond to significant differences ($p < 0.05$) among the samples. C (black column): phycocyanin content of *A. platensis* F&M-C256 biomass before thermal treatment.

incorporated with EVO oil. The samples with EVO oil incorporation treated for 10 min always presented the highest residual content of phycocyanin ($p < 0.05$), followed by the biomasses with EVO oil treated for 20 min. After prolonged thermal treatment (40 min) at high temperature (160 °C) *A. platensis* F&M-C256 biomass integrated with EVO oil still contained 0.43g/100g phycocyanin, corresponding to 5.3g/100g of the phycocyanin supplied with the microalgal biomass, i.e., three times more than the corresponding sample without EVO oil. The findings related to the degradation of phycocyanin with temperature are in agreement with the results reported by several authors (Antelo et al., 2008; Böcker et al., 2019; Chaiklahan et al., 2012). Chaiklahan et al. (2012) found that incubation at temperatures between 47 and 64 °C rapidly decreased the concentration and half-life of phycocyanin in solution. Hence, besides EVO oil addition, in order to avoid phycocyanin degradation, the selection of the most suitable combination of time and temperature for thermal treatment must be carefully considered.

3.2. Effect of extra virgin olive oil against phycocyanin degradation in heat-treated phycocyanin powder

To verify the protective effect of EVO oil against phycocyanin degradation, the same thermal treatments (three temperatures 50, 100 and 160 °C and three times 10, 20 and 40 min) as for *A. platensis* F&M-C256 biomass were carried out on phycocyanin powder, incorporated or not with EVO oil. Three-way ANOVA was carried out in order to investigate the effect of the three considered parameters. The phycocyanin content (g/100g) of heat-treated phycocyanin powder incorporated or not with EVO oil are presented in Fig. 3.

The interactions temperature-time and temperature-EVO oil addition resulted statistically significant ($p < 0.0001$), while the other interactions did not affect the residual phycocyanin content in phycocyanin powder after thermal treatment (Supplementary Table 2). In agreement with the results on *A. platensis* F&M-C256 biomass (Fig. 1), a dependence between phycocyanin degradation, temperature, and time of exposure was found (Fig. 3). Indeed, with increasing time of thermal treatment, the residual content of phycocyanin decreased to different extents. Moreover, the EVO oil addition reduces the degrading effect of higher temperature of treatment. The percentage difference between samples treated for 40 min with EVO oil incorporation and without was 65% at 160 °C, 24% at 100 °C, and 22% at 50 °C. The same phenomenon

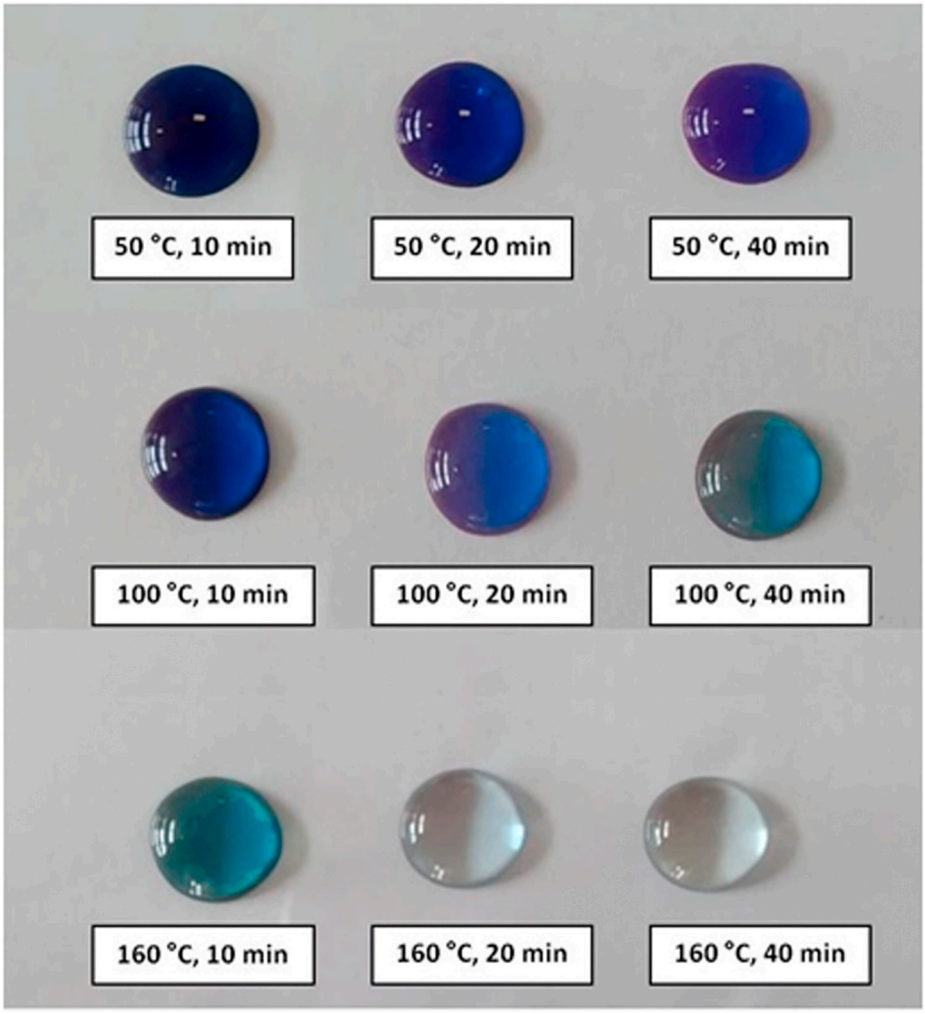

Fig. 2. (color) Drops of phycocyanin extracted from *A. platensis* F&M-C256 biomass without EVO oil after thermal treatment at 50, 100 and 160 °C for 10, 20 and 40 min.

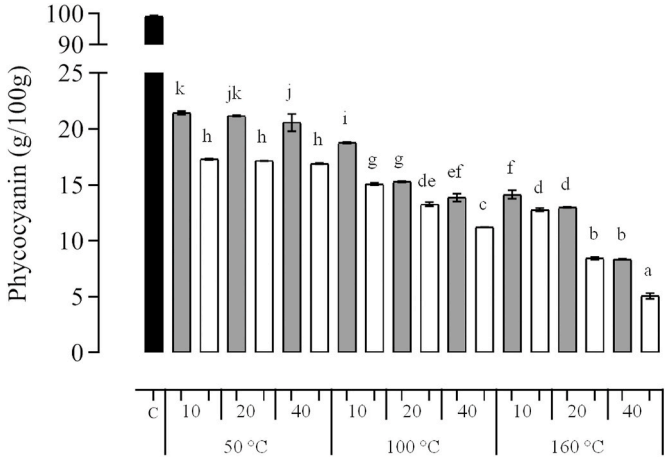

Fig. 3. Phycocyanin content (g/100g) in phycocyanin powder incorporated with EVO oil (grey columns) and phycocyanin powder without EVO oil (white columns) after thermal treatment at 50, 100, and 160 °C for 10, 20 or 40 min. Results are expressed as average ± standard deviation (n = 3). Different letters correspond to significant differences ($p < 0.05$) among the samples. C (black column): phycocyanin content in phycocyanin powder before thermal treatment.

was observed for the samples treated for 10 and 20 min.

3.3. Effect of pure tocopherol against phycocyanin degradation in heat-treated A. platensis F&M-C256 biomass

In order to evaluate if tocopherol contained in EVO oil was the responsible for the protective action, a trial, limited to the thermal treatment at 160 °C, in which *A. platensis* F&M-C256 biomass was mixed with pure α-tocopherol was also carried out. The phycocyanin content (g/100g) of biomasses not incorporated and incorporated with different concentrations of tocopherol are presented in Fig. 4.

Three concentrations were investigated: 0.5 mg α-tocopherol/100g of *A. platensis* F&M-C256 biomass, 1.5 mg α-tocopherol/100g of *A. platensis* F&M-C256 biomass (about the same concentration of tocopherol present in "crostini" and derived from EVO oil), and 2.5 mg α-tocopherol/100g of *A. platensis* F&M-C256 biomass.

Two-way ANOVA showed a high statistical significance ($p < 0.0001$) for the interaction time-tocopherol content (as well as for the single factors). In fact, the protective effect of tocopherol changed to a different extent with increasing time of thermal treatment (Fig. 4). After each thermal treatment, *A. platensis* F&M-C256 biomass incorporated with α-tocopherol at all concentrations always presented a higher phycocyanin content compared to the biomass without α-tocopherol (not exceeding 170 mg phycocyanin/100g biomass). These results are in agreement with the data of the residual phycocyanin content reported in

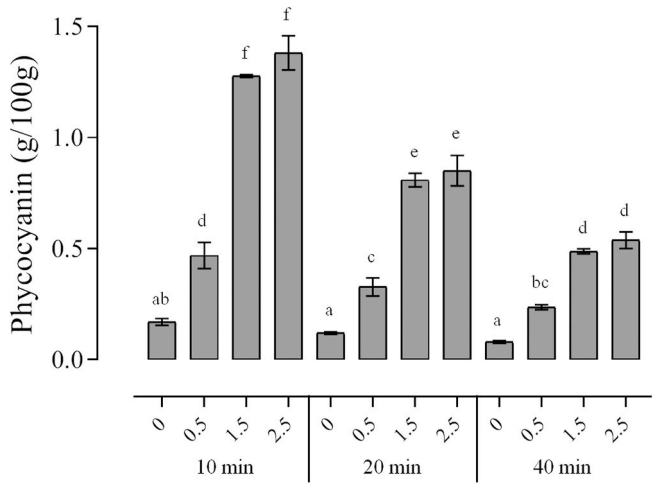

Fig. 4. Phycocyanin content (g/100g) of *A. platensis* F&M-C256 biomass incorporated with different concentrations of tocopherol. Three concentrations were investigated: 0.5 mg α-tocopherol/100g of biomass, 1.5 mg α-tocopherol/100g of biomass, and 2.5 mg α-tocopherol/100g of biomass. As control (0) biomass without α-tocopherol was also investigated. All biomass samples were heat-treated at 160 °C for 10, 20, and 40 min. Results are expressed as average ± standard deviation (n = 3). Different letters correspond to significant differences ($p < 0.05$) among the samples.

Fig. 1 at 160 °C. For all the durations of thermal treatment, the highest level of protection was detected with 1.5 and 2.5 mg α-tocopherol/100g of biomass. Moreover, at the same treatment time, no significant difference between the phycocyanin content in biomass incorporated with 1.5 and 2.5 mg α-tocopherol/100g was detected, suggesting a probable saturation effect of tocopherol addition, independently of the time of thermal treatment.

Considering the ratio between tocopherol (derived from EVO oil or directly added) and phycocyanin in biomass, it is possible to observe an identical protective effect. The addition of EVO oil to the *A. platensis* F&M-C256 biomass led to a ratio between tocopherol (from EVO oil) and phycocyanin of 1 mg of tocopherol per 5.1 g of phycocyanin. When tocopherol was added to the biomass at the concentrations of 0.5, 1.5, and 2.5 mg/100g of biomass, the resulting ratios were 1 mg of pure tocopherol per 16.2, 5.4, and 3.2 g of biomass respectively. Given the similar tocopherol:biomass ratio, the residual phycocyanin contents (g/100g of biomass) in biomasses incorporated with EVO oil and in those enriched with 1.5 mg tocopherol/100g biomass at 160 °C were not statistically different for each duration of the thermal treatment.

Tocopherols are well known to be important natural antioxidants for the oxidative stability of vegetable oils (Arora, Bagoria, & Kumar, 2010) and of pork patties (Haak et al., 2009). Estévez, Ventanas, and Cava (2005) found that the addition of rosemary essential oil (6g/100g), containing tocopherols and tocotrienols, in refrigerated frankfurters inhibited protein oxidation by 22.8%. Salminen et al. (2006) investigated the effect of protein containing by-products of deoiling processes rich in phenolics, such as rapeseed meal, camelina meal, and soy flour, in inhibiting oxidation of lipids and proteins in cooked pork meat patties. The authors reported that the difference in the antioxidant activity of camelina and rapeseed toward oxidation of proteins might partly be explained by the difference in the tocopherol content. Therefore, especially for meat products, tocopherol demonstrated to be effective in proteins protection from oxidation and degradation phenomena occurring during cooking and storage.

3.4. Phycocyanin degradation in A. platensis F&M-C256 "crostini" incorporated with butter, extra virgin olive oil and sunflower oil

After testing the action of EVO oil as protecting agent against phycocyanin degradation in spirulina biomass and phycocyanin powder, the protective effect of different lipid matrix incorporation for a potential application in a baked product,"crostini", was evaluated. Phycocyanin content of "crostini" (Fig. 5A) enriched with 6g/100g *A. platensis* F&M-C256 biomass and incorporated with butter, EVO oil and sunflower oil was determined (Fig. 5). Sunflower oil and butter were chosen due to their common application in the bakery industry.

After cooking, *A. platensis* "crostini" incorporated with 10g/100g EVO oil presented 440 mg phycocyanin/100g "crostini" and, thus about 90% of the phycocyanin present in the microalgal biomass incorporated in "crostini" (486 mg phycocyanin/100g "crostini"). A similar amount of phycocyanin was found for *A. platensis* F&M-C256 "crostini" incorporated with 10g/100g sunflower oil (450 mg phycocyanin/100g "crostini") (Fig. 5C). Even if sunflower oil contains a higher tocopherol content than EVO oil, its thermal protective effect upon phycocyanin in "crostini" is similar. It is probable that after a certain critical α-tocopherol concentration, sufficient to protect phycocyanin, a saturation effect occurred. "Crostini" incorporated with 12.5g/100g butter showed a lower content of phycocyanin (160 mg phycocyanin/100g "crostini") compared to "crostini" with vegetable oils. The lowest phycocyanin amount was determined in control "crostini" that presented only 100 mg phycocyanin/100g "crostini", thus about 20% of the initial phycocyanin from the microalgal biomass (486 mg phycocyanin/100g "crostini").

A low phycocyanin content for control "crostini" and butter "crostini" (drops practically colourless) compared to those integrated with oils (presence of light blue color in the drops) was observed (Fig. 5B). Furthermore, macroscopically it is also possible to note that no difference in terms of phycocyanin content between EVO oil "crostini" and sunflower oil "crostini" is present (drops with the same intensity of light blue colour) (Fig. 5B).

The use of phycocyanin in food and other applications is limited due to its sensitivity to thermal treatment, which results in protein degradation, loss of bioactive potential and fading of the blue color (Chaiklahan et al., 2012).

In a previous work we have shown that phycocyanin in spirulina enriched "crostini" was not degraded after cooking (Niccolai, Venturi, et al., 2019), while in cookies phycocyanin content decreased significantly after cooking (Batista et al., 2017). The main difference between cookies and "crostini" production was the use of a different lipid matrix: reduced-fat margarine and EVO oil, respectively. Since reduced-fat margarine generally contains a lower amount of α-tocopherol, up to 5 mg/100g (Rader, Weaver, Patrascu, Ali, & Angyal, 1997), than EVO oil, the obtained results seem to confirm a protective action of tocopherol against phycocyanin degradation during cooking.

Several studies also highlighted the limited stability of phycocyanin and that only moderate increase in protein thermal stability has been achieved by adding preservatives (Antelo et al., 2008; Chaiklahan et al., 2012; Jespersen, Strømdahl, Olsen, & Skibsted, 2005; Martelli et al., 2014; Mishra et al., 2008). Antelo et al. (2008) found that the addition of the protein stabilizer sorbitol between 10 and 50g/100g in a treatment at 62 °C for 30 min increased the half-life values of phycocyanin extract, proving that its decolorization after thermal treatment was related to degradation of the protein chain. Other researchers evaluated encapsulation techniques or stabilizing effects through the addition of sugars (Chaiklahan et al., 2012; Martelli et al., 2014) or ascorbic acid (Mishra et al., 2008). However, to the best of our knowledge, the protective action of vegetable oils (such as EVO oil or sunflower oil) against phycocyanin degradation during cooking of bakery products has not been so far investigated.

4. Conclusions

Temperature and time affect the phycocyanin degradation during thermal treatment, both for *A. platensis* F&M-C256 biomass and phycocyanin powder; interestingly, the addition of EVO oil strongly protects phycocyanin from this phenomenon. In *A. platensis* -based

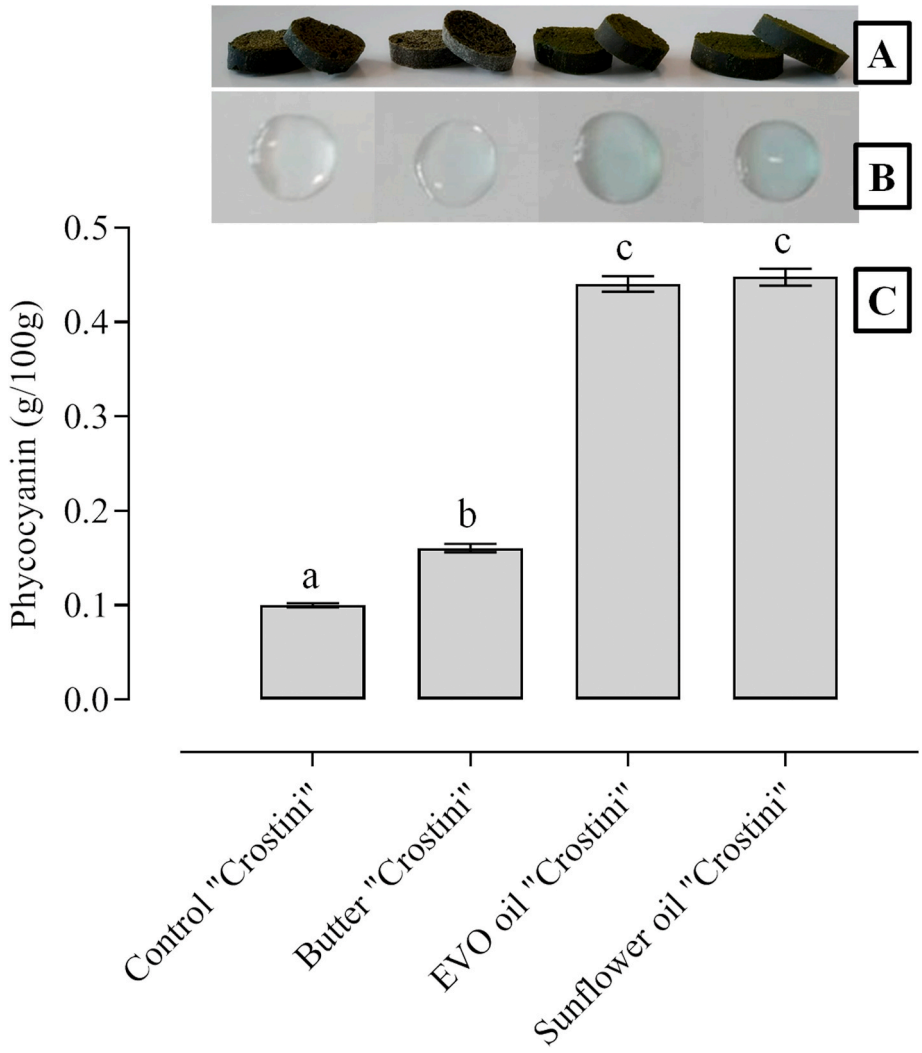

Fig. 5. (**color**) *A. platensis* F&M-C256-based "crostini" (A), drops of phycocyanin extracted from "crostini" (B), and phycocyanin content (g/100g) of "crostini" enriched with 6g/100g *A. platensis* F&M-C256 biomass without lipid matrix (control "crostini") or incorporated with butter, extra virgin olive (EVO) oil or sunflower oil (C). Results are expressed as average ± standard deviation (n = 3). Different letters correspond to significant differences ($p < 0.05$).

"crostini" production, the incorporation of EVO oil or sunflower oil at the concentration of 10g oil/100g of dough has been shown to be effective in protecting phycocyanin (up to 90%) from thermal degradation during cooking. Probably tocopherol contained in EVO and sunflower oils is the main responsible for the protective action against phycocyanin degradation. Further studies should be carried out in order to maximize the proper vegetable oil amount to add to "crostini" dough, taking into account the baked product recipe. Overall, the incorporation of EVO or sunflower oils into the dough is a simple and useful tool for all those bakery industries that intend to use *A. platensis* biomass and/or phycocyanin as a natural food coloring and bioactive component for the production of functional bakery goods avoiding pigment degradation.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Alberto Niccolai: Formal analysis, Writing - original draft. **Manuel Venturi:** conception and design. **Viola Galli:** conception and design. **Niccolò Pini:** conception and design.

Declaration of competing interest

M.R. Tredici and L. Rodolfi have a financial interest in F&M S.r.l. M. Venturi has a financial interest in FoodMicroTeam S.r.l. The other authors declare no competing interests with respect to the work described in this manuscript.

Acknowledgments

The authors are indebted to Azienda Agricola Serenissima S.S. (Conche di Codevigo, Padova, Italy) for kindly providing the *A. platensis* biomass. AN holds a fellowship funded by the POR FSE 2014–2020 – Progetto Strategico "STREAMING", sottoprogetto PhotoWING (grant number: UNIFI_FSE2017, Regione Toscana, Italy).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2020.110776.

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