Formulation and stability of food-grade hydrogels from natural polymers containing extracted C-Phycocyanin from *Spirulina platensis*

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For several years, industry has shown great interest in *Spirulina platensis* due to its many potential beneficial properties (antioxidant, anti-inflamatory etc). However, lately there is a growing interest for the blue-protein C-Phycocyanin (C-PC) existing in this algae. C-PC belongs to the phycobiliprotein family and is the photosynthetic blue-protein of *Sp. platensis* and the major phycobiliprotein of this algae, constituting up to 20% of its dry weight. Nowadays, the stabilization of C-PC for use as a natural blue colorant is of particular commercial interest, due to the advantages that it offers. Firstly, the use of natural colorants has gained the attention of food and pharmaceutical industry since the synthetic colorants commonly used have been associated with various side effects such as cancer development or induction of allergic reactions [1]. Additionally, an important benefit of C-PC is that it provides high protein yield by relatively easy extraction procedures. It is water-soluble and easily detected by photometric methods, having an absorption maximum at 610-620nm. Nevertheless, C-PC's bright blue color is stable in a short range of pH values and the protein degrades with heat or light exposure [2].

Hydrogels, solid or semi-solid systems consisting of at least two components, one of which is a liquid and in particular water [3], are appealing to food industry due to their special characteristics (water-holding capacity, texture, biocompatibility etc), offering the development of innovative products with features such as stability in different temperatures, acidity conditions etc.

The aim of this study is to combine the natural colorant C-PC with biocompatible, non-toxic hydrogels from natural polymers in order to increase the color stability of C-PC in various conditions adequate for food products/procedures.

The study consists of three parts: the extraction of C-PC from crude *Sp. platensis*, the formulation of hydrogels and encapsulation of C-PC in the polymer network and finally the stability study. After retrieving C-PC-containing extract in the form of a blue powder from crude *Sp. platensis*, it gets encapsulated in the polymer network of a hydrogel prepared based on chitosan and another one based on hydroxypropyl methylcellulose (HPMC). The proposed matrices are non-toxic, biocompatible and food-grade.

The stability of the encapsulated C-PC was studied and all systems tested have shown stability over time. Moreover, the effect of the presence of sugars and preservatives as well as the effect of temperature on the gel stability was examined in various solvents.

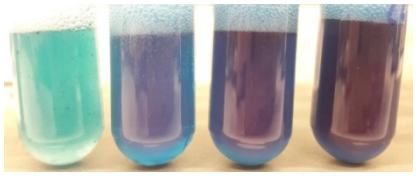

Figure 1. Different concentrations of C-PC-containing extraction in HPMC based hydrogel

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[1]G. Martelli, C. Folli, L. Visai, M. Daglia and D. Ferrari, *Process Biochemistry*, 2014, 49(1), 154-159.
[2]L. Jespersen, L. D. Strømdahl, K. Olsen and L. H. Skibsted, *European Food Research and Technology*, 2005, 220(3-4), 261-266.
[3]M. Zoumpanioti, H. Stamatis and A. Xenakis, *Biotechnology Advances*, 2010, *28*(3), 395-406.

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1. Introduction

The **aim of this study** is to combine the natural colorant C-Phycocyanin (C-PC) with biocompatible, non-toxic hydrogels from natural polymers in order to increase the color stability of C-PC in various conditions adequate for food products/procedures.

Hydrogels, are solid or semi-solid systems consisting of at least two components, one of which is a liquid and in particular water [1].

Advantages of natural polymers:

- ✓ Eco-friendly source
- ✓ High holding capacity
- ✓ Biodegradability
- ✓ Non-toxic [2]

C-Phycocyanin (C-PC)

- Protein of approximately 20 kDa , consisting of two subunits (a and b)
- Belongs to the phycobiliprotein family
- Photosynthetic blue-protein of *Sp. Platensis*
- Major phycobiliprotein of this algae, constituting up to 20% of its dry weight
- Biliproteins are found assembled in large, distinct granules—phycobilisomes—that are considered analogous to the light-harvesting complexes containing chlorophyll in green plants

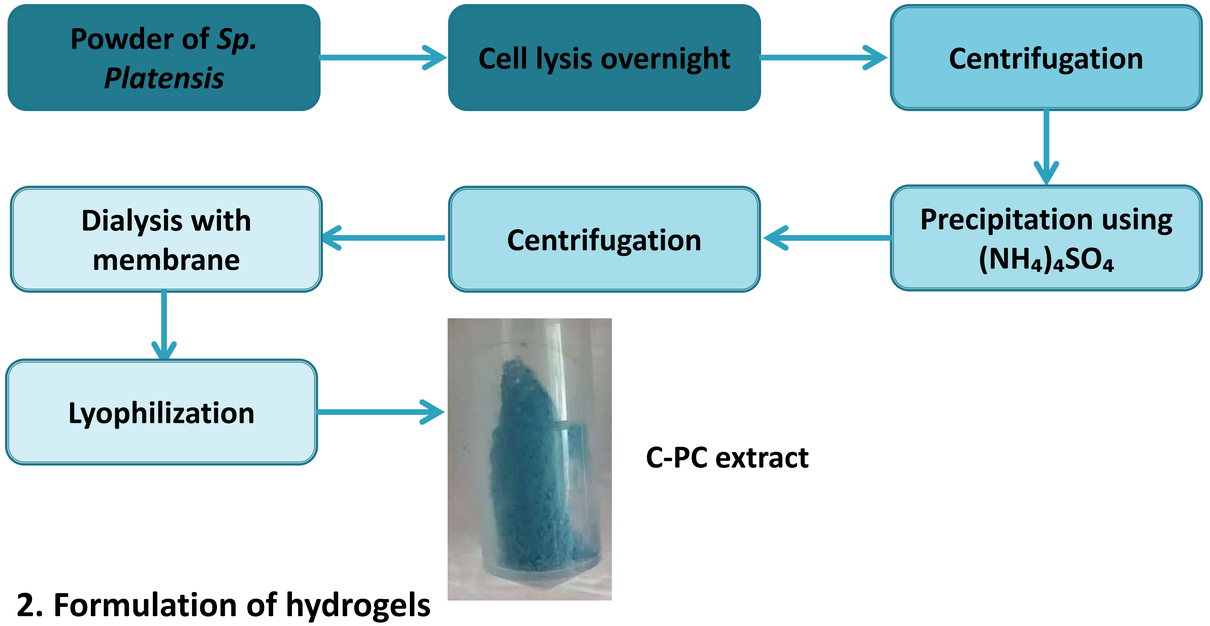
Advantages of C-PC as a blue colorant:

- ✓ Natural-does not appear the side effects of the synthetic ones [3]
- ✓ Water-soluble
- ✓ Easily detected by photometric methods (610-620nm)
- ✓ Provides high protein yield by relatively easy extraction procedures

HOWEVER bright blue color is stable in a short range of pH values and the protein degrades with heat or light exposure [4].

2. Methodology

1. Extraction of C-PC from crude *Sp. Platensis*



2. Formulation of hydrogels

Three systems were formulated, using various polymers and solvents:

i. Chitosan + Acetic Acid 1% (pH 3)
ii. Hydroxypropyl methylcellulose (HPMC) + Acetic Acid 1% (pH 3)
iii. HPMC + water

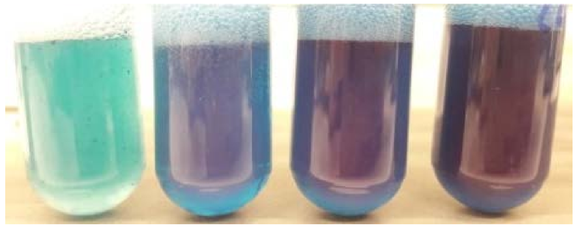
3. Encapsulation of C-PC in the polymer network

After the preparation of the hydrogels C-PC-extract was added and the encapsulation was performed with mild stirring

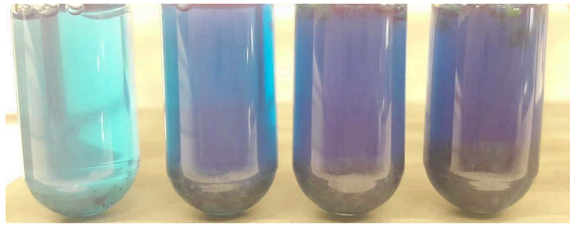
4. Stability study

3. Concentration, pH & temperature test

a) Concentration

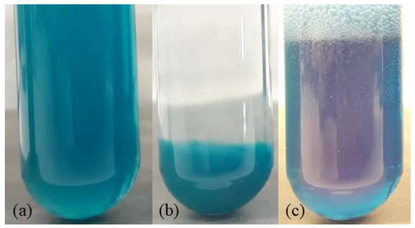


10, 20, 30 and 40 mg of C-PC-containing extraction in HPMC based hydrogel



10, 20, 30 and 40 mg of C-PC-containing extraction in Chitosan hydrogel

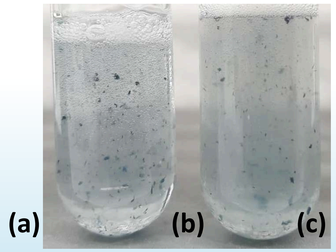
b) pH



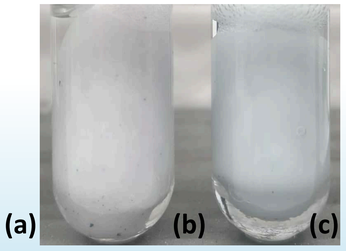
C-PC-containing extraction in (a)Acetic Acid 1% Day 0, (b) Acetic Acid 1% Day 2, (c) Hydrogel system HPMC with Acetic Acid 1% Day 7

c) Temperature

The samples were heated in water bath fist at 50 ^{o}C and then at 80 ^{o}C for 30 min in presence and absence of fructose



Chitosan hydrogel containing C-PC-extract
(a)before heating
(b)after 30 min incubation without fructose
(c)after 30 min incubation with fructose



HPMC hydrogel containing C-PC-extract
(a)before heating
(b)after 30 min incubation without fructose
(c)after 30 min incubation with fructose

Conclusions

- ✓ Extraction of C-PC from *Sp. platensis* powder was performed successfully using biocompatible buffers throughout the entire procedure
- ✓ Non-toxic, biocompatible and food-grade hydrogel systems based on Chitosan and Hydroxypropyl methylcellulose were developed
- ✓ C-PC was successfully encapsulated inside the polymer network
- ✓ All three systems showed stability over time and retained the bright blue color of C-PC
- ✓ The systems and the blue color of C-PC remained stable after heating at 50 ^{o}C for 30 mins
- ✓ After 30 mins heating at 80 ^{o}C the HPMC system collapsed, while the chitosan system remained stable. However, the color of C-PC faded significantly in all systems.
- ✓ The hydrogels containing fructose maintained slightly more of the C-PC blue color

References

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