

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23

Supplementary Material

The Generally Recognized as Safe (GRAS) Microalgae *Haematococcus pluvialis* Wet as a Multifunctional Additive for Coloring and Improving the Organoleptic and Functional Properties of Foods

Aly Castillo^{1,2,3*}, Tiane C. Finimundy^{4,5}, Sandrina A. Heleno^{4,5}, Paula Rodrigues^{4,5}, Filipa A. Fernandes^{4,5}, Simón Pereira⁶, Marta Lores², Lillian Barros^{4,5}, Carmen Garcia-Jares^{1,2}

¹CRETUS, Department of analytical Chemistry, Nutrition and Food Science, Universidade de Santiago de Compostela, E-15782, Santiago de Compostela, Spain.

²LIDSA, Department of Analytical Chemistry Nutrition and Food Science, Universidade de Santiago de Compostela, E-15782, Santiago de Compostela, Spain.

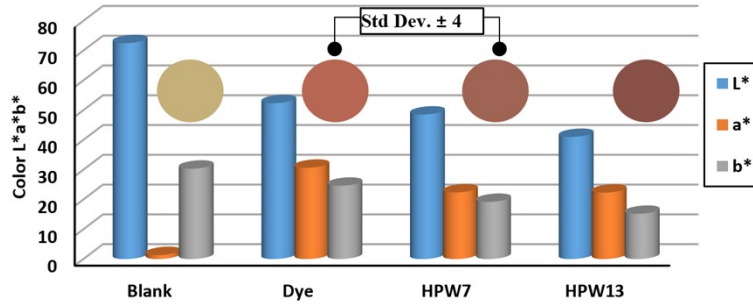
³i-Grape Laboratory, Edificio Emprendia, Avda Mestre Mateo s/n, 15702, Santiago de Compostela, Spain

⁴Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, 5300-253, Portugal.

⁵Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança. Campus de Santa Apolónia, 5300-253 Bragança, Portugal.

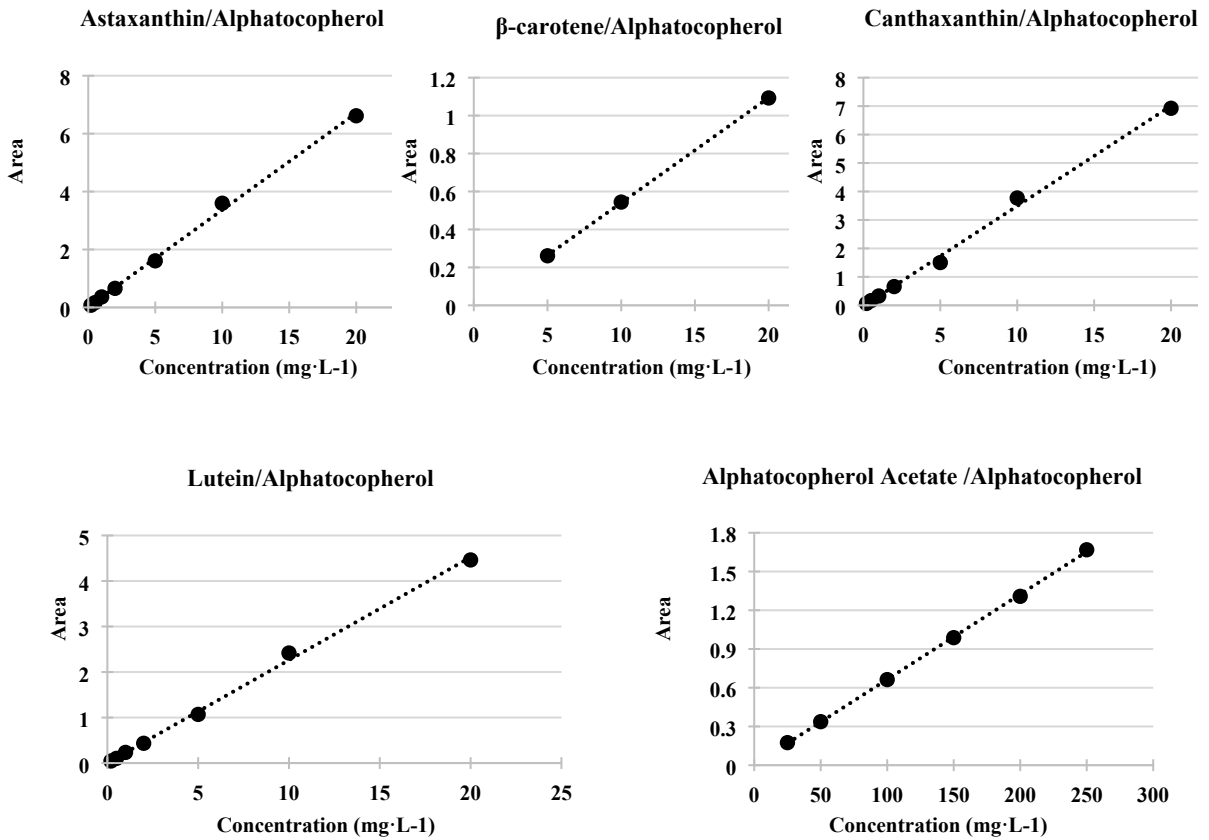
⁶Institute of Aquaculture and Department of Microbiology and Parasitology, Universidade de Santiago de Compostela, Campus Vida, E-15782, Santiago de Compostela, Spain.

Corresponding author: AC. alyjesus.castillo.zamora@usc.es

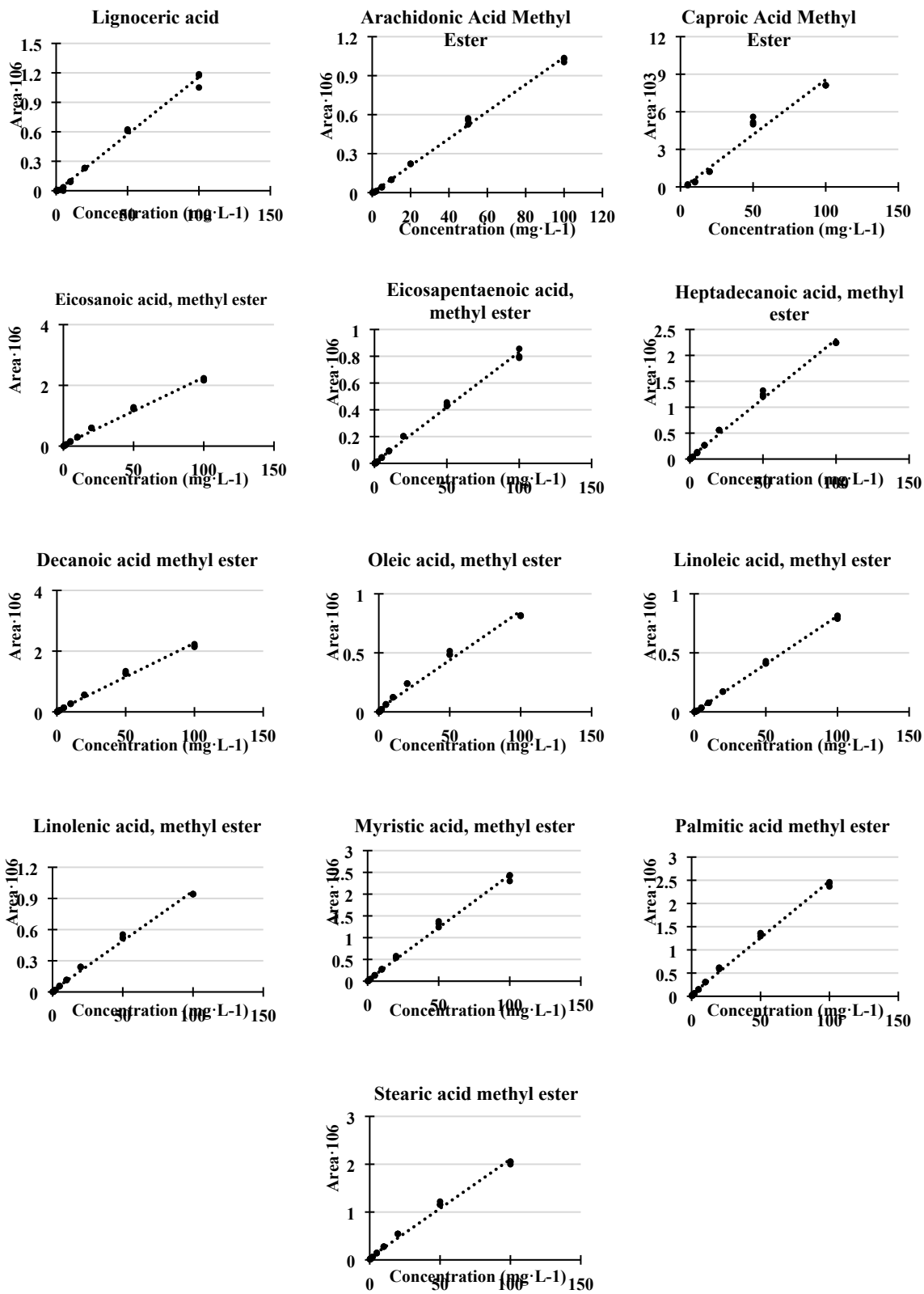


25

26 **Figure S1.** Comparative analysis of the color profile (Lab) of the *filloas*. Blank: control
 27 group; Dye: addition of synthetic colorant; HPW7 and HPW13: addition of *H. pluvialis* wet
 28 at concentrations of 7% and 13% ($\text{g}_{\text{microalgaDW}} \cdot \text{g}^{-1}_{\text{flour}}$) respectively.



29 **Figure S2.** Calibration curves of the carotenoid, internal and subrogate standards by HPLC-
 30 DAD used for the quantification of the *filloas* samples. Linearity ranges, equation of the
 31 curve and R^2



32 **Figure S3.** Calibration curves of the fatty acids standards by GC-MS used for the
 33 quantification of the *filloas* samples. Linearity ranges, equation of the curve and R²

34 **Table S1.** Respective chemical composition of the analyzed commercial dye

Function	E Code	Compound	CAS	Formula
Colorant	E122	Azorubine	3567-69-9	C ₂₀ H ₁₂ N ₂ Na ₂ O ₇ S ₂
	E102	Tartrazine	1934-21-0	C ₁₆ H ₉ N ₄ Na ₃ O ₉ S ₂
Common additives in both dyes				
Acidulant	E330	Citric Acid	77-92-9	C ₆ H ₈ O ₇
Preservative	E202	Potassium Sorbate	24634-61-5	C ₆ H ₇ O ₂ K

35

36 **Table S2.** Studied compounds in *filloa* samples for characterization and quantification of
37 their bioactive profile. CAS numbers, purity, and suppliers

Acronym	Common name	CAS	Purity (%)	Molecular Formula	Company
CAROTENOID					
	Astaxanthin	472-61-7	98.1	C ₄₀ H ₅₂ O ₄	¹ BIOSYNTH
	Lutein	127-40-2	98.9	C ₄₀ H ₅₆ O ₂	² BIOPURIFY
	Canthaxanthin	514-78-3	98.2	C ₄₀ H ₅₂ O ₂	³ EHRENSTORFER
	β -Carotene	7235-40-7	99.7	C ₄₀ H ₅₆	⁴ ALFA
FATTY ACIDS					
C10:0	Decanoic	334-48-5	99.5	C ₁₀ H ₂₀ O ₂	⁵ SIGMA
C14:0	Myristic	544-63-8	99.7	C ₁₄ H ₂₈ O ₂	⁶ TCI
C16:0	Palmitic	57-10-3	99.9	C ₁₆ H ₃₂ O ₂	⁷ FLUOROCHEM
C17:0	Heptadecanoic	506-12-7	98.7	C ₁₇ H ₃₄ O ₂	⁸ GLENTHAM
C18:0	Stearic	57-11-4	99.4	C ₁₈ H ₃₆ O ₂	⁶ TCI
C18:1	Oleic	112-80-1	99.6	C ₁₈ H ₃₄ O ₂	⁵ SIGMA
C18:2	Linoleic	60-33-3	99.2	C ₁₈ H ₃₂ O ₂	⁵ SIGMA
C18:3	Linolenic ($\gamma+\alpha$)	463-40-1	98.5	C ₁₈ H ₃₀ O ₂	⁵ SIGMA
C20:0	Arachidic	506-30-9	99.9	C ₂₀ H ₄₀ O ₂	⁴ ALFA
C20:5	Eicosapentaenoic	10417-94-4	98.3	C ₂₀ H ₃₀ O ₂	¹ BIOSYNTH
INTERNAL STANDARD					
C19:0	Nonadecanoic Acid	646-30-0	99.5	C ₁₉ H ₃₈ O ₂	⁶ TCI
C22:0	Lignoceric Acid	557-59-5	98.9	C ₂₄ H ₄₈ O ₂	⁶ TCI
	Alpha tocopherol	59-02-9	97.0	C ₂₉ H ₅₀ O ₂	⁵ SIGMA
	Alpha tocopherol acetate	58-95-7	99.1	C ₃₁ H ₅₂ O ₃	⁴ ALFA

38 ¹ Biosynth Carbosynth (BIOSYNTH) (West Berkshire, England); ² Biopurify Phytochemicals Ltd.
39 (BIOPURIFY) (Sichuan, China); ³ Dr. Ehrenstorfer (Augsburg, Germany); ⁴ Alfa Aesar (Karlsruhe,
40 Germany); ⁵ Sigma-Aldrich Chemie GmbH (SIGMA) (Steinheim, Germany); ⁶ Tokyo Chemical Industry
41 (TCI) (Tokyo, Japan); ⁷ Fluorochem Ltd. (FLUOROCHEM) (Derbyshire, England); ⁸ Glentham Life Sciences
42 Ltd. (GLENTHAM) (Wiltshire, England)

43