### Accepted Manuscript

Title: Novel protocol for lutein extraction from microalga *Chlorella vulgaris* 

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| PII:           | S1369-703X(17)30170-5                           |
|----------------|---|
| DOI:           | http://dx.doi.org/doi:10.1016/j.bej.2017.06.019 |
| Reference:     | BEJ 6741  |
| To appear in:  | Biochemical Engineering Journal                 |
| Received date: | 2-3-2017  |
| Revised date:  | 1-5-2017  |
| Accepted date: | 29-6-2017                                       |

Please cite this article as: Martina D'Este, Davide De Francisci, Irini Angelidaki, Novel protocol for lutein extraction from microalga Chlorella vulgaris, Biochemical Engineering Journalhttp://dx.doi.org/10.1016/j.bej.2017.06.019

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## ACCEPTED MANUSCRIPT

#### Novel protocol for lutein extraction from microalga Chlorella vulgaris

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#### Highlights

- A novel method for the extraction of lutein from microalgae was developed.
- Water was replaced by ethanol in the saponification step.
- Saponification and extraction were conducted simultaneously.
- Quantity of lutein extracted increased 3 folds compared to conventional methods.
- Final lutein purity increased from 73.6% to 93.7%.

#### ABSTRACT

Lutein is a pigment generally extracted from marigold flowers. However, lutein is also found in considerable amounts in microalgae. In this study a novel method was developed to improve the extraction efficiency of lutein from microalga *C. vulgaris*. Differently from conventional methods, ethanol was used instead of water in the saponification step, which was conducted simultaneously to the solvent extraction, performed using dichloromethane. The amount of lutein extracted from *C. vulgaris* dried biomass increased more than threefold, from  $0.20 \pm 0.00$  mgLutein/gDM to  $0.69 \pm 0.08$  mgLutein/gDM. Lutein purity was increased from 73.6% to 93.7% by decreasing the ethanol-water ratio from 85% to 50% in the resolubilization step. The novel method was also tested with tetrahydrofuran. The extraction proved to be again more effective than the conventional one; however

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