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Astaxanthin production by *Xanthophyllomyces dendrorhous* growing on a low cost substrate

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Abstract The aim of the study was to evaluate the new approaches for the reduction of food wastes, promoting industrial by-products and food waste recovery. The possibility, starting from fruit and vegetable waste (FVW), of producing astaxanthin from *Xanthophyllomyces dendrorhous*, in fermentative process using a batch mode was evaluated and the effect of light intensity on carotenoid biosynthesis from the yeast, has been investigated. Astaxanthin is a red oxygenated carotenoid valuable in different industries such as aquaculture, nutraceutical, pharmaceutical, and food. The results obtained in this study highlight that the growth and carotenogenesis of *X. dendrorhous* on FVW and on the synthetic medium, used as reference, were not suggestively different, neither for yeast growth, being 6.2 g/l and 5.3 g/l, nor for astaxanthin production, resulting 410 μg/g and 355 μg/g, respectively. This preliminary research study and show the chance for reducing waste and pollution from food and agro-industrial wastes discussing the opportunity of producing astaxanthin from *X. dendrorhous* using low-cost substrates.

Keywords Astaxanthin · Carotenoids · Waste · Recovery · Feed · Food

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Introduction

Nowdays, the growth in food waste, deriving from agro-industries and household consumption, and its management turn into serious issues affecting our modern society not only from an economically and environmental point of view but mainly for of its negative effects on global sustainability (Paritosh et al. 2017).

The Food and Agriculture Organization (FAO) reported that about the 30% of total production is wasted globally every year (Gustavsson et al. 2013). According the European Union (EU), annually in the EU, 88 million tons, equivalent to 173 kg/person, of wastage from food are produced (European Commission 2016).

The possibility of taking advantage of industrial and agricultural origin food waste have become increasingly important to environment health, business economy and growth.

The use of food waste materials, in order to obtain interesting molecules to address to cosmetic, nutraceutical or feed field became interesting and promising, considering the high potential of biomolecules, that often remain or that could be obtained from these waste. The importance of natural biomolecules has been extensively showed (Aghraz et al. 2018; Benameur et al. 2018; Pluchtová et al. 2018; Alesci et al. 2015; Di Stefano et al. 2015; Mikusova et al. 2010). In this framework the possibility of formulating culture media for microorganisms growth, could achieve to obtain great economic advantage not only for new obtained products but also for the problems associated with the disposal. Culture media from food waste could be used for the biotechnological production of carotenoids from algae, bacteria or yeast.

The family of carotenoids comprise of 600 isoprenoid pigments that have several beneficial proprieties for human and animal health (Thies et al. 2017).

In 2017 the worldwide market of carotenoids was esteemed at $1.5 billion, and it could reach $2 billion in 2022 (Research and Markets 2018). The principal carotenoids market value is represented by astaxanthin (24%). Among the carotenoids, astaxanthin is one of the most interesting ones for its wide range of possible applications.

To date, several natural sources of astaxanthin have been explored because of the growing demand for novel applications, ranging from pharmaceutical and cosmetic industry to food, nutraceutical and feed industries (Liu et al. 2018; Santini et al. 2017; Del Campo and García-González 2007; Naviglio et al. 2007). This ketocarotene is used largely in aquaculture, as feed additive for the pigmentation of salmonid meat and crustacean shells (Industry experts 2015; Mata-Gómez et al. 2014).

The main part of the carotenoids market (about 76%) is represented by products obtained from the chemical industries; however, nowadays several studies are addressed to the extraction of carotenoids from food waste and to their production from microorganisms.

Astaxanthin production from algae is higher than the one obtained from yeasts (Lorenz and Cysewski 2000), anyway the last one is preferred because of the higher growth rates and easier cultivation conditions (Bumbak et al. 2011) that, at an industrial scale, might decrease the production time (Mata-Gómez et al. 2014).

Several previous researches investigated the possibility of using waste deriving from food and agro-industries, for its potential as raw material in fermentation processes for Single Cell Protein or carotenoid production for animal feed (Gervasi et al. 2018a, b; Galanakis 2015; Tropea et al. 2013).

The present research investigates the possibility of culturing *Xanthophyllomyces dendrorhous* on fruit and vegetable waste (FVW) as culture medium, and examines the influence of the irradiation on the carotenoid biosynthesis (Table 1).

Materials and methods

Microorganism and medium

Xanthophyllomyces dendrorhous ATCC 24202 purchased from American-Type Culture Collection was used in this study. A culture medium obtained from fruit and vegetable waste (FVW) collected from large-scale distribution was used. FVW were washed and blended; the filtered juice was boiled, centrifuged and sterilized by autoclaving before being used at 10% as culture medium. The test was also carried out on a synthetic culture medium (SC) containing (g/l) glucose, 20; $(NH_4)_2SO_4$, 5; KH_2PO_4, 1; SO_4^{2-}, 7; H_2O 0.5; $CaCl_2$ $2H_2O$, 0.1; yeast extract, 3; peptone, 5; pH was 5.05 without any further correction.

Inoculum preparation

From a slant culture a loop of *X. dendrorhous* ATCC 24202 was inoculated into a 200 ml Erlenmeyer flask containing SC and grown at 22 °C on a rotary shaker at

Table 1 Culture media obtained from food waste (%, w/w on dry matter)

pH	5.0
Total soluble solid	21.9 brix
Total sugar	134.34 g/L
Reducing sugar	52.71 g/L

250 rpm for ca. 48 h to an OD_{600} of 0,8. An amount of this culture (10%) was used for the inoculum.

Culture conditions

The microorganism was cultivated, in double, by using FWV and SC media, in 2L Erlenmeyer flasks, with a working volume of 1L, at 22 °C on a rotary shaker at 250 rpm, with a period of 7 days under illumination, performed by using a Philips TLD 18 W/54 lamp. Further tests were carried out under the same conditions, as described above, except for the illumination.

Analytical methods

The FVW medium was analyzed. The determination of residual reducing sugars was conducted according the 3,5-dinitrosalicylic acid (DNS) method of Miller (1959). The total sugar was measured using the phonol–sulfuric method (Dubois et al. 1956).

The microorganism samples (5 ml) was harvested by centrifugation (10 min at 8000 g, rt) double washed, filtered through a 0.45 µm Millipore pre-weighted filter and used for determination of pellet percent dry weight by weighing the pellet before and after drying at 60 °C for 24 h.

Xanthophyllomyces dendrorhous astaxanthin was extracted, from samples-lyophilized after the harvesting conducted as reported before, by suspending it in DMSO at 55 °C for 5 min, then adding 0.5 ml of 0.01 M Na_3PO_4 and then 4 ml of hexane/ethyl acetate (1:1, v/v). Astaxanthin was determined by a UFLCXR liquid chromatograph combined with an LCMS-8040 (Shimadzu, Kyoto, Japan) equipped with a 100 × 2.1 mm, 1.7 µm C_{18} column as reported in Gervasi et al. (2018b).

Results

The goal of the study was to investigate the possibility of using low cost substrate for the production of astaxanthin by *X. dendrorhous.* In line with this objective, FVW and SC media were compared.

Low-cost raw materials, for astaxanthin production through fermentation process using *X. dendrorhous*, has been widely explored, infact these substrates contain good amount of natural carbon sources that have been proved to promote astaxanthin synthesis (Rodríguez-Sáiz et al. 2010). The total sugar contained in FVW were used by *X. dendrorhous* (data not reported).

As expected, the highest yeast growth and astaxanthin production, by using FVW as culture medium, were observed when the fermentation test were carried out under continuous illumination with white light. The importance of the carbon source in this process is a significant factor to consider together with the influence of the light. The metabolism of the microorganism depend on the quantity and the typology of the carbon source available, instead the carotenogenesis is positively affected by the light; infact the microorganisms produce carotenoids in order to prevent themselves from damage caused from the light.

The same test was conducted in SC, used as reference medium; these tests did not show a significant difference underling the possibility of using low cost substrate as media for astaxanthin production form *X. dendrorhous*. While the recorded values about the growth of *X. dendrorhous* and its astaxanthin production didn't show a high and important dissimilarity when the two culture media where used, instead the presence of the light makes a big difference for the carotenogenesis.

The results, reported in Figs. 1 and 2, showed the yeast grow and the produced carotenoids on FVW at 10% and SC media after 7 days of fermentation under continuous white light and in dark condition

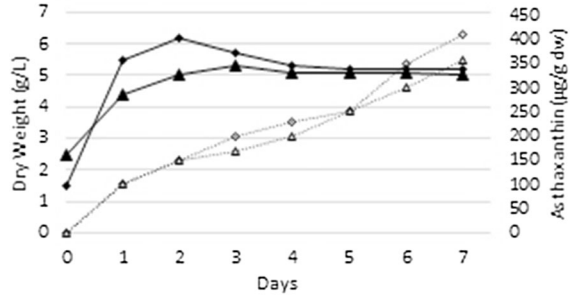

Fig. 1 *X. dendrorhous* growth and carotenoids production in a batch system on FVW and SC media at a constant temperature of 22 °C and stirrer speed of 200 rpm in an enlightened system. Notes: filled square growth on SC medium, filled triangles growth on FVW medium in a enlightened system. Open square and triangles, astaxanthin production on SC and FVW media respectively. Results represent the yeast dry weight and carotenoid amount and are the mean of triplicate assays ± error bars

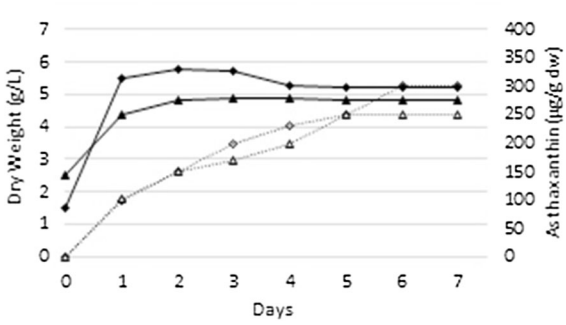

Fig. 2 *X. dendrorhous* growth and carotenoids production in a batch system on FVW and SC media at a constant temperature of 22 °C and stirrer speed of 200 rpm in a dark system. Notes: filled square growth on SC medium, filled triangles growth on FVW medium in a dark system. Open square and triangles, astaxanthin production on SC and FVW media respectively. Results represent the yeast dry weight and carotenoid amount and are the mean of triplicate assays ± error bars

respectively. The growth of *X. dendrorhous*, increased from 1.49 to 6.2 g/l and from 2.5 to 5.3 g/l when cultivated on SC and FVW respectively. The highest astaxanthin amount was reordered in both the tests at the 7 days, reaching 410 µg/g and 355 µg/g on SC and FVW respectively. The results confirm that FVW at 10% and pH 5.0 was a good substrates for *X. dendrorhous* growth and carotenogenesis. Several test were carried out to assess these conditions (data not reported).

In Fig. 2 the influence of the light for the carotenogenesis is shown. The carotenoid production enhancement of has been widely described in several microorganisms, such as in *X. dendrorhous* (Bhosale 2004; de la Fuente et al. 2010). It has been shown that light exposure has a negative influence on *Xanthophyllomyces* growth but promote carotenoids, particularly astaxanthin, production (An and Johnson 1990a, b).

Conclusions

Carotenoids represent very interesting molecules, being used as nutraceutical, colorants and supplements in feed and food and in cosmetic or pharmaceutical industries (Santini et al. 2017; Santini and Novellino 2017). Astaxanthin is very important in aquaculture for the pigmentation of salmonids and crustacean. At the industrial scale. *X. dendrorhous* is one of the principal microorganisms useed for microbial astaxanthin production. Several previously studies showed the possibility of using agro industrial food as culture media for astaxanthin production from *X. dendrorhous* (Amado and Vázquez 2015).

From the tests conducted, considering the interaction between the yeast and the substrate, the result obtained were not suggestively different, neither for yeast growth nor for astaxanthin production.

The study conducted by using FVW revealed the possibility of valorizing this material by producing astaxanthin from *X. dendrorhous* in a fermentation process in batch mode under continuous illumination, being the white light a fundamental requisite for improving carotenoids production (Gervasi et al. 2018b).

This preliminary study confirm the capacity food waste as substrate for the production of astaxanthin, which has several application from pharmaceuticals and cosmetic industries to nutraceutical, food, and feed fields. This last is the most conspicuous sector of astaxanthin applications (Saini et al. 2018).

The principal propose of these study is oriented to the minimization, prevention, reuse and recycling of waste, considering that, in the waste stream of many agro-industries and in food wastage, many biomolecules and compound that could be valorized are still present (Salvo et al. 2017a, b; Gervasi et al. 2016; Alesci et al. 2015; Rotondo et al. 2011).

Compliance with ethical standards

Conflict of interest Authors declare that they have no conflict of interest.

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