Biochemical Composition of *H. pluvialis*

Because of the unique life cycle of *H. pluvialis*, cellular composition of this microalga varies tremendously between its "green" and "red" stages of cultivation (Table 1).

TABLE 1

Composition content (% of DW)	Green stage	Red stage
Proteins	29–45	17–25
Lipids (% of total)	20–25	32–37
Neutral lipids	59	51.9–53.5
Phospholipids	23.7	20.6–21.1
Glycolipids	11.5	25.7–26.5
Carbohydrates	15–17	36–40
Carotenoids (% of total)	0.5	2–5
Neoxanthin	8.3	n.d
Violaxanthin	12.5	n.d
β -carotene	16.7	1.0
Lutein	56.3	0.5
Zeaxanthin	6.3	n.d
Astaxanthin (including esters)	n.d	81.2
Adonixanthin	n.d	0.4
Adonirubin	n.d	0.6
Canthaxanthin	n.d	5.1
Echinenone	n.d	0.2
Chlorophylls	1.5–2	0

Adapted from Grewe and Griehl (2012). n.d., no data.

Table 1. Typical composition of *H. pluvialis* biomass in green and red cultivation stages.

Protein

In green stage, during favorable growth conditions most *H. pluvialis* strains are rich in protein (29–45) (Table 1), lower protein content (23.6%) have been however observed in a Bulgarian strain *Haematococcus* cf. *pluvialis* Rozhen-12 during green stage cultivation (Gacheva et al., 2015). It was estimated that proteins contribute to 21 (Kim et al., 2015) and 23% (Lorenz, 1999) of cellular content during red stage cultivation of *H. pluvialis*. Amino acid composition of proteins in the red stage indicated that proteins were mainly composed of aspartic acid, glutamic acid, alanine, and leucine with total amino acid content of 10.02/100 mg, 46.0% of which belonged to essential amino acids (Lorenz, 1999; Kim et al., 2015).

Carbohydrates

In green stage, carbohydrate content approximates to 15–17%, about a half of the red stage (Table 1). In the red stage, under conditions of stress (e.g., nutrient starvation, light stress, high acidity, temperature variations etc.), *H. pluvialis* accumulates higher content of carbohydrates (starch), for example 38 (Lorenz, 1999), 60 (Recht et al., 2012), and 74% (Boussiba and Vonshak, 1991). Under prolonged stress conditions starch is consumed in the cell.

Lipid

In green stage, total lipid content varies from 20 to 25%, with approximately 10% lipids composed predominantly of short (C16, C18) polyunsaturated fatty acids deposited in the chloroplasts. Neutral lipids are predominant lipid class in both green and red cells (Table 1). In red stage, prolonged stress conditions direct larger flux toward the synthesis of neutral lipids—triacylglycerols (TAG). Red stage cells can accumulate up to 40% of their cell weight as cytoplasmic lipid droplets (LD), and considerable amount of secondary metabolites including up to 4% of the ketocarotenoid astaxanthin (Boussiba et al., 1992, 1999; Saha et al., 2013). The phospholipid content does not change compared to the green stage, while the glycolipid fraction nearly doubles in red cells when compared with green vegetative cells (Damiani et al., 2010). The total fatty acid profile of *H. pluvialis* is relatively complex. Palmitic (16:0), linoleic (18:2), and linolenic (18:3) acids are predominant components of the profile with highly polyunsaturated species also present in considerable amounts (Table 2). Based on the comparative studies on fatty acids profile of two different *H. pluvialis*, it was revealed that both strains varied in composition, especially of palmitic (16:0), oleic (18:1), linoleic (18:2), and linolenic (18:3) acids. This variation might be associated with several factors such as culture environment, stress conditions, culture parameters, variation of strain origin, nutrient etc. Higher lipid content of *H. pluvialis* grown under nutrient starvation and the suitable profile of its fatty acids indicate a possibility of biodiesel production from this microalga (Damiani et al., 2010; Saha et al., 2013). The massive astaxanthin accumulation in *H. pluvialis* is a cellular response to stress conditions and is accompanied by the enhanced biosynthesis of triacylglycerols (TAG) (Zhekisheva et al., 2002, 2005; Cerón et al., 2007), and the reduction in photosynthetic activity of PSII, loss of cytochrome *f*, and subsequent reduction in electron transport, and increased respiration rate (Boussiba, 2000). During transition from green vegetative cells to red aplanospores after exposure to stress conditions astaxanthin start to accumulate as fatty acid mono- or diesters in cytoplasmic lipid droplets (LD) (Aflalo et al., 2007). As cells undergo transition to red stage, both chlorophyll and protein contents drop.

TABLE 2

Fatty acids	*Haematococcus* sp. KORDI03 (Kim et al., 2015)	*H. pluvialis* (Lorenz, 1999)
C12:0 lauric	N/A	0.1
C14:0 myristic	0.1	0.5
C15:0 pentadecanoic acid	0.1	N/A
C16:0 palmitic	13.7	29.0
C16:1 palmitoleic	0.5	0.6
C16:2	0.4	N/A
C16:3	3.5	N/A
C16:4	3.3	N/A
C17:0 margaric	N/A	0.2
C17:1 margaroleic	N/A	1.3
C18:0 stearic	0.7	2.1
C18:1 oleic	4.9	25.9
C18:2 linoleic	24.9	20.8
C18:3 linolenic	39.7	12.8
C18:4 octadecatetraenoic	5.8	1.4
C20:0 arachidic	N/A	0.6
C20:1 gadoleic	0.5	0.3
C20:2 eicosadenoic	N/A	1.2
C20:3 eicosatrienoic gamma	N/A	0.5
C20:4 arachidonic	0.9	1.4
C20:5 eicosapentaenoic	0.6	0.6
C22:0 behenic	N/A	0.4
C24:0 lignoceric	0.3	0.2
C24:1 nervonic acid	0.1	0.1
$\sum$ SFAs	15.0	33.2
$\sum$ MUFAs	6.0	28.1
$\sum$ PUFAs	79.1	38.7
Total	100.0	100.0

Table 2. Comparison of fatty acid composition (%) of two different *H. pluvialis* strains.

Carotenoid

The carotenoid fraction of green vegetative cells consists of mostly lutein (75–80%), β-carotene (10–20%) and others, including chlorophyll a and b, primary carotenoids, violaxanthin, neoxanthin, lactucaxanthin, and zeaxanthin (Renstrøm et al., 1981; Harker et al., 1996a). In the red stage, the total carotenoid content is markedly enhanced, and the characteristic primary carotenoid pattern of vegetative stage is replaced by secondary carotenoids, mainly astaxanthin (80–99% of total carotenoids) (Harker et al., 1996a; Dragos et al., 2010). The ratio of carotenoids to chlorophylls is about 0.2 in the green stage and increases in the red stage by an order of magnitude and reaches about 2–9. The majority of astaxanthin is not deposited in its free form but it exists within the cell as fatty acid esters of astaxanthin, usually mono- or diesters of palmitic (16:0), oleic (18:1), or linoleic (18:2) acids. This type of modification is required for the deposition of this highly polar molecule within non-polar matrix of lipid droplets. Approximately 70% monoesters, 25% diesters, and only 5% of the free ketocarotenoid is present in the mature "red" cells of *H. pluvialis* (Zhekisheva et al., 2002; Solovchenko, 2015). Under certain conditions of stress *H. pluvialis* has been shown to accumulate up to 3–5% DW of astaxanthin (Han et al., 2013; Chekanov et al., 2014).

H. pluvialis-derived Astaxanthin

H. pluvialis as a Major Source of Astaxanthin

H. pluvialis can accumulate up to 5% DW of astaxanthin and is considered as the best natural source of this high-value carotenoid pigment (Wayama et al., 2013). Dietary supplements containing *Haematococcus* astaxanthin has proved to be safe to humans and widely used for over 15 years as a nutraceutical supplement with no adverse side-effects of its supplementation (Capelli and Cysewski, 2013; Yang et al., 2013). Natural astaxanthin from *H. pluvialis* or krill oil is available in the market as a dietary supplement in dosages from 3.8 to 7.6 mg per day due to potential health benefits (Yang et al., 2013). As societies nowadays are looking toward “green” solutions, natural astaxanthin form *H. pluvialis* seems to be more favorable than its synthetic counterpart due to structure, function, application, and security (Choubert and Heinrich, 1993; Capelli and Cysewski, 2013; Pérez-López et al., 2014).