Heliyon 7 (2021) e06085

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Two-level factorial analysis of the effect of fructose on DHA biosynthetic capacity of *Aurantiochytrium* sp. SW1

Vidyah Manikan [a], Yusuf Nazir [b], Aidil Abdul Hamid [a,*]

[a] Department of Biological Sciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia 43600 UKM Bangi, Selangor, Malaysia
[b] Department of Food Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia

ARTICLE INFO

Keywords:
Thraustochytrids
Fructose
Factorial design
Docosahexaenoic acid (DHA)

ABSTRACT

Thraustochytrids are getting increasingly popular due to their high potential role as alternative producers of the high-valued ω-3 polyunsaturated fatty acids (PUFA), docosahexaenoic acid (DHA). While most thraustochytrids prefer glucose as the major carbon source, few strains have been reported to prefer fructose. One such strain is *Aurantiochytrium* sp. SW1. In this study, the effect of fructose on DHA accumulation by SW1 was investigated using a two-level full factorial design. Besides, biomass, lipid and DHA accumulation profiles of SW1 cultivated in fructose and glucose media were compared. Results revealed that fructose has a very significant positive effect on the volumetric DHA content. Meanwhile, its involvement in affecting DHA biosynthetic capacity, though significant, is not very profound. It was also found that when cultivated in fructose medium, SW1 had a less steep log phase compared to that of glucose medium. However, after 48h of cultivation, biomass and lipid accumulation in fructose medium outweighed the other. Volumetric DHA content in fructose medium at 96h was 11% higher than that of glucose medium. Overall, fructose was found to be a more suitable substrate for biomass, lipid and DHA accumulation in SW1 compared to the conventional source, glucose.

1. Introduction

Omega-3 fatty acids, in the past decades, have been widely interrelated to healthy aging due to the many fundamental roles they play in human health and development. Docosahexaenoic acid (DHA) is an important member of the ω-3 family. It is an essential component in the brain, skin and eyes [1,2]. This fatty acid is typically acquired from fatty fish such as salmon, mackerel and swordfish, crustaceans such as crab and spiny lobster, as well as mollusks such as scallop and oyster [3].

Ever since the introduction of the concept of "designer oils" [4], bioproduction of DHA from microalgae has gained great interest over the conventional method of obtaining it from seafood. The idea of acquiring DHA from microalgae instead of seafood-based sources is advantageous. Besides better purification potential, microalgal DHA is usually free from chemical contamination [5]. Unlike depleting fish stock that is incapable to provide adequate supply for the continually increasing market demand [6], bioproduction of DHA from microalgae promises a continuous supply that can be adjusted depending on the demand.

Thraustochytrids are unicellular heterokont microalgae, having an extensive geographical distribution from the polar to tropical regions, inhabiting various environments like mangrove sediments, estuaries and deep-sea ecosystems [7]. Lacking chloroplasts, they are unable to perform photosynthesis but possess excellent DHA synthesis capabilities which make them considered to be a primary DHA producer in marine environments [8]. Thraustochytrids have suitable properties for the industrial production of DHA, i.e., they can be mass cultured using a jar fermenter without sunlight, and they can accumulate large amounts of DHA in well-developed intracellular lipid droplets.

Several strains of thraustochytrids have been reported to produce high biomass, containing large amounts of DHA-rich lipid [9]. One such thraustochytrid is *Aurantiochytrium* sp. SW1, which has been reported to accumulate up to half of its biomass as lipid, containing 40-60% DHA from the total fatty acids. This isolate, unlike most other DHA-producing thraustochytrids, prefers fructose over glucose for biomass, lipid and DHA accumulation [10]. Utilization of this unique characteristic of SW1 is expected to lead to betterment in overall DHA productivity. As previous studies only report on the basis of general production parameters such as concentration and yield, in this study, we intend to define the significance of fructose as the major carbon source for DHA production using a two-level full factorial design. Compared to the conventional 'one factor at a time' (OFAT) method, the factorial design offers a wider inductive basis, where it covers a broader space from which inferences

* Corresponding author.
E-mail address: aidilmikrob@gmail.com (A.A. Hamid).

https://doi.org/10.1016/j.heliyon.2021.e06085
Received 21 November 2020; Received in revised form 11 December 2020; Accepted 20 January 2021

could be drawn. Besides, analysis using this method enables the determination of the magnitude of the effect of fructose on its own as well as its interaction with other medium components. This provides the key to understanding the overall effects [11] of all medium components on DHA production in SW1.

2. Materials and ethods

2.1. Microorganism and culture conditions

Aurantiochytrium sp. SW1 (GenBank: KF500513, UNiCC UPM-WDCM 988: UPMC 963) was obtained from the Microbial Physiology Laboratory, School of Biosciences and Biotechnology, Universiti Kebangsaan Malaysia. Glucose (60 g/L) was replaced with fructose (60 g/L) for the preparation of seed cultures involving production cultures containing fructose. Cultivation was carried out in 500 mL Erlenmeyer flasks at 30 °C.

2.2. Experimental design

A two-level full factorial design was employed to investigate the effects of fructose and other components present in the production medium. Design expert software (DOE; version 10.0, Stat-Ease, USA) was used to design the experiments. The ranges of the medium components are shown in Table 1. The ranges were chosen based on preliminary experiments (data not shown).

The design of the experiment together with the response values are shown in Table 2. The design contained a duplicate for each run, summing up to a total run number of 32. The confidence level was set at 95%.

2.3. Analytical methods

Culture conditions and analytical methods for the determination of biomass, lipid, and DHA concentrations are as described in our previous work [12].

3. Results

In our previous studies, experimentations to investigate the ability of SW1 to utilize different carbon sources showed that fructose is the most preferred sugar for biomass, lipid, and DHA accumulation based on the levels of DHA concentration and yield achieved [10]. Similar results were observed by Chatdumrong et al. [13] where *S. limacinum* BR2.1.2 produced an enhanced amount of DHA when fructose was used as a major carbon source replacing glucose. Therefore, in this study, a two-level full factorial design was employed to further establish the significance of fructose on DHA accumulation with a particular emphasis on its interactions with other medium components. For better evaluation of the impact of fructose, DHA accumulation was represented as volumetric content (g/L culture) as well as DHA biosynthetic capacity of the cells (% g/g dry biomass). Results of the experiment are shown in Table 2.

3.1. Effects of fructose on volumetric content (g/L) of DHA

The effect of fructose, as well as other factors on the volumetric DHA production, was initially visualized by the significance of each coefficient, indicated by the F and *p* values from the analysis of variance (ANOVA) data (Table 3). Values of 'Prob > F' less than 0.005 indicate that the model is significant and vice versa. Based on Table 3, the p-value of the model was ($p < 0.0001$) and the F-value was large (562.77) implying that the model was significant to interpret the data. "Lack of Fit p-value" was 0.8475 indicating the Lack of Fit was not significant relative to the pure error. The ANOVA showed that all the above-listed factors and interactions, except factor C (MSG), are highly significant, with a probability value less than 0.0001.

Besides, it is important to determine the relative effect of fructose and its interaction with other variables to determine the extent of its participation in the effect of the volumetric output of DHA by SW1. Thus, the changes in response values or term as "standardized effects "were demonstrated in Figure 1. This value functions as an indicator of the magnitude of effect contributed by a factor as well as its direction, whether it is a positive or negative effect. The summary of the effects of all medium components and their interactions on the volumetric content of DHA is illustrated in Figure 1.

Figure 1 noticeably shows that factors A (fructose) and B (yeast extract), as well as their interaction, exert very significant positive effects on the DHA content compared to other factors. Similarly, interactions between AC (fructose-MSG) and CD (MSG-sea salt) affect DHA content on a considerable negative scale, whereas AD (fructose-sea salt) and BD (yeast extract-sea salt) contribute small negative effects. As for 3-factor interactions, BCD (yeast extract-MSG-sea salt) exert visible negative effect, meanwhile ABC (fructose-yeast extract-MSG), ABD (fructose-yeast extract-sea salt) and ACD (fructose-MSG-sea salt) exerts positive effect. Overall, based on Figure 1, fructose corresponds to three-quarters of the four most impactful factors (A, B, AB and ACD). This indicates the significant positive role played by fructose among the rest of the medium components in boosting the volumetric DHA content of SW1. This is due to fructose, being the carbon source, is a critical compound in the biosynthesis of fatty acids.

3.2. Effects of fructose on DHA biosynthetic capacity (% g/g biomass) of SW1

ANOVA results on the effects of fructose on DHA biosynthetic capacity (% g/g biomass) of SW1 were indicated in Table 4. The result showed that factors B, AB, AC, AD, BD, CD, ACD and BCD are the highly significant factors with probability value of less than 0.0001; meanwhile, factors A, C and ABD score probability value of 0.0181, 0.0007 and 0.0025 respectively. Even though the role of fructose in promoting DHA biosynthetic capacity of SW1 is less prominent compared to that of DHA content, it can still be concluded that fructose does have a significant positive effect based on its involvement in the very significant interactions, AB and ACD.

Similarly, the standardized effects of the medium components and their interactions on the DHA biosynthetic capacity of SW1 were also evaluated (Figure 2). In contrast to volumetric DHA content discussed in the previous section, the biosynthetic capacity, which reflects the amount of DHA capable to be synthesized per gram of biomass, is affected approximately equally in both positive and negative directions by the medium components and their interactions (Figure 2).

Fructose, while appearing to be a negative factor in contrast to its effect on the volumetric content of DHA, exerts a very small effect on the DHA biosynthetic capacity of SW1. However, its interaction with yeast extract (AB) and MSG-sea salt (ACD) have a profound positive effect.

Table 1. Range of medium components for two-level factorial analysis.

Factors	Low level (-1) (g/L)	High level (+1) (g/L)
A: Fructose	0	60
B: Yeast extract	0	2
C: MSG	0	8
D: Sea salt	0	6

Table 2. Experimental design and response values for two-level factorial analysis.

Std order	Fructose	Yeast extract	MSG	Sea salt	DHA (g/L)		DHA biosynthetic capacity (% g/g biomass)	
					Predicted	Actual	Predicted	Actual
1	-1	-1	-1	-1	0.44	0.46	15.80	15.81
2	-1	-1	-1	-1		0.46		15.75
3	1	-1	-1	-1	1.61	1.45	24.03	25.35
4	1	-1	-1	-1		1.74		22.75
5	-1	1	-1	-1	0.22	0.19	7.97	7.88
6	-1	1	-1	-1		0.20		8.10
7	1	1	-1	-1	3.28	3.15	29.03	29.15
8	1	1	-1	-1		3.08		28.87
9	-1	-1	1	-1	1.17	1.07	24.74	23.41
10	-1	-1	1	-1		1.23		26.11
11	1	-1	1	-1	1.15	1.19	18.11	18.00
12	1	-1	1	-1		1.14		18.18
13	-1	1	1	-1	2.00	2.12	30.47	31.83
14	-1	1	1	-1		1.91		29.07
15	1	1	1	-1	3.04	3.54	27.00	26.92
16	1	1	1	-1		3.27		27.12
17	-1	-1	-1	1	1.36	1.38	20.28	20.54
18	-1	-1	-1	1		1.31		19.97
19	1	-1	-1	1	1.15	1.19	11.60	11.63
20	1	-1	-1	1		1.15		11.62
21	-1	1	-1	1	1.19	1.23	32.59	32.54
22	-1	1	-1	1		1.18		32.69
23	1	1	-1	1	3.85	3.45	32.29	32.50
24	1	1	-1	1		3.26		32.04
25	-1	-1	1	1	1.09	1.17	23.78	22.24
26	-1	-1	1	1		1.04		25.37
27	1	-1	1	1	1.18	1.12	12.08	12.71
28	1	-1	1	1		1.20		11.40
29	-1	1	1	1	0.69	0.73	16.25	16.59
30	-1	1	1	1		0.62		15.86
31	1	1	1	1	3.84	3.91	22.62	22.09
32	1	1	1	1		3.81		23.19

Table 3. ANOVA on the effect of fructose and other factors on volumetric content (g/L) of DHA.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	87.39	12	7.28	562.77	<0.0001 (significant)
A-Frc	31.80	1	31.80	2457.50	<0.0001
B-YE	24.33	1	24.33	1879.84	<0.0001
C-MSG	6.125E-004	1	6.125E-004	0.047	0.8301
D-Salt	0.30	1	0.30	23.51	0.0001
AB	24.12	1	24.12	1863.70	<0.0001
AC	1.45	1	1.45	112.33	<0.0001
AD	0.83	1	0.83	64.30	<0.0001
BD	0.71	1	0.71	54.72	<0.0001
CD	1.58	1	1.58	122.43	<0.0001
ABC	0.33	1	0.33	25.67	<0.0001
ACD	1.13	1	1.13	86.94	<0.0001
BCD	0.81	1	0.81	62.32	<0.0001
Residual	0.25	19	0.013		
Lack of Fit	0.012	3	3.921E-003	0.27	0.8475 (Not significant)
Pure Error	0.23	16	0.015		
Cor Total	87.63	31			

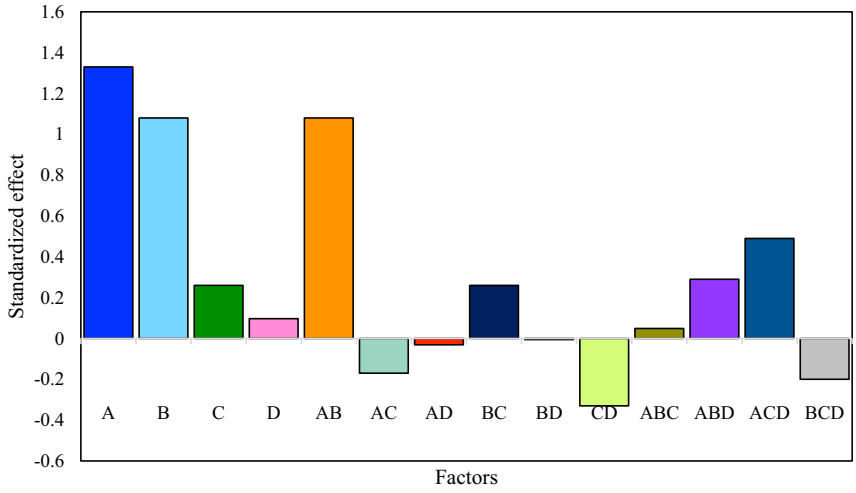

Figure 1. Effects of medium components and their interaction on the volumetric content of DHA.

Table 4. ANOVA on the effect of fructose and other factors on DHA biosynthetic capacity.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	1743.73	14	124.55	118.56	<0.0001 significant
A-Frc	2.98	1	2.98	2.83	0.1106
B-YE	285.61	1	285.61	271.86	<0.0001
C-MSG	0.26	1	0.26	0.25	0.6234
D-Salt	4.00	1	4.00	3.81	0.0676
AB	225.14	1	225.14	214.31	<0.0001
AC	159.76	1	159.76	152.07	<0.0001
AD	140.28	1	140.28	133.53	<0.0001
BC	19.69	1	19.69	18.74	0.0005
BD	73.33	1	73.33	69.80	<0.0001
CD	259.12	1	259.12	246.65	<0.0001
ABD	13.68	1	13.68	13.02	0.0022
ACD	231.45	1	231.45	220.31	<0.0001
BCD	281.44	1	281.44	267.89	<0.0001
ABCD	47.00	1	47.00	44.73	<0.0001
Residual	17.86	17	1.05		
Lack of Fit	0.017	1	0.017	0.015	0.9030 not significant
Pure Error	17.84	16	1.12		
Cor Total	1761.59	31			

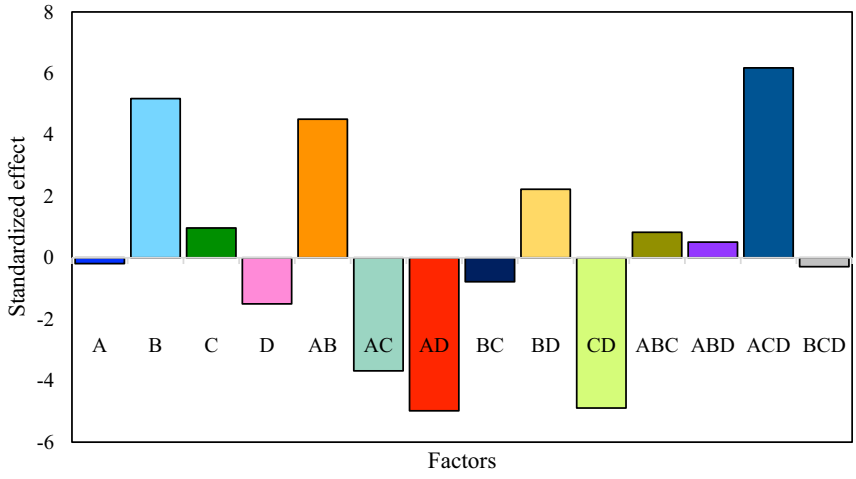

Figure 2. Effects of medium components and their interactions on the DHA biosynthetic capacity of SW1.

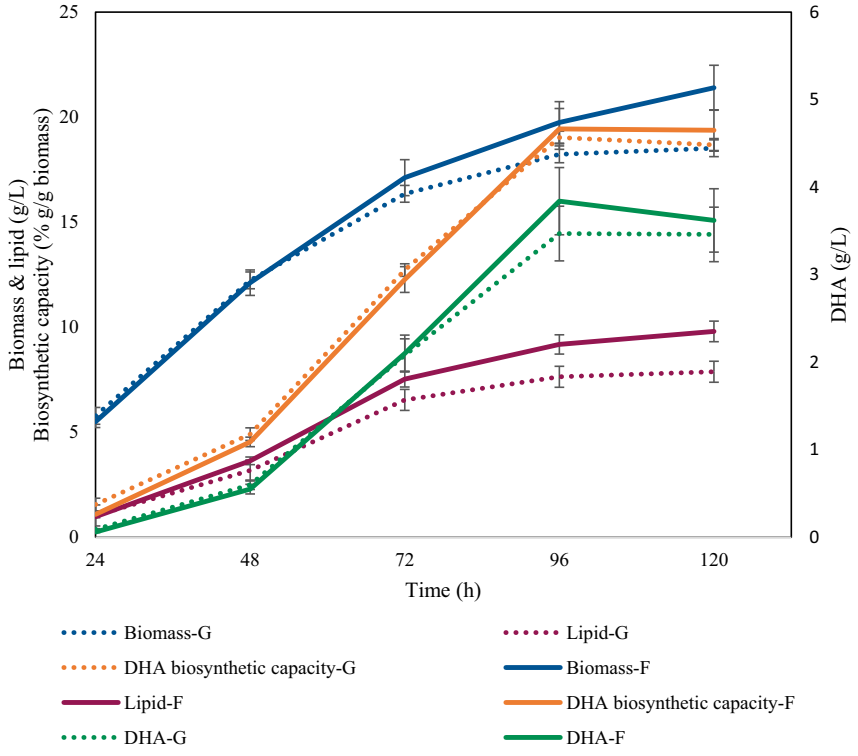

Figure 3. Biomass, lipid and DHA accumulation profiles of SW1 cultivated in medium containing glucose compared to fructose; G = glucose, F = fructose.

3.3. Growth, lipid and DHA accumulation profiles of SW1

Figure 3 illustrates the biomass, lipid and DHA accumulation profiles of SW1 cultivated separately in media containing glucose and fructose. Based on the chart, cultures which had fructose, though with a marginally less steep log phase (24–48h), results in noticeably higher biomass and lipid accumulation at 72h onwards. As for volumetric DHA content, 3.84 g/L was achieved using fructose, which was 11% higher than that of glucose. However, only negligible differences were observed in terms of DHA biosynthetic capacity between the two cultures throughout the cultivation period.

Therefore the increment in the volumetric DHA content was a result of the increase in biomass in the culture which was supplied with fructose as the major carbon source. These results are in good agreement with the observations in the two-level factorial experiments discussed above.

4. Discussion

Being a very attractive and promising material for the sustainable production of DHA, thraustochytrids are studied extensively in order to improve market prospects. Many different strategies are utilized to enhance and engineer the lipid production of these organisms [14,15]. Of such, impacts of the composition of growth media are vastly studied and literature is profuse about it [16]. Fructose, along with other monosaccharides, especially glucose, as well as polysaccharides such as glycerol, is assimilated well by various strains of *Aurantiochytrium* and *Schizochytrium*. Nevertheless, the assimilation capacities of each substrates differ among strains and few strains have been reported to have less preference over fructose compared to the other substrates. Table 5 lists a few examples of thraustochytrids and their fructose assimilation capabilities.

In our previous studies, intended to evaluate the use of glucose as the major carbon source for DHA accumulation, it was found that DHA concentration in total lipid was only affected significantly by glucose-yeast extract and glucose-MSG interactions. In line with this study, the single factor and interaction of fructose-yeast extract were also shown to poses a strong impact on the volumetric DHA production of SW1 (Figure 1). This could be related to their vital functions in the lipid biosynthesis pathway and growth. An increment in the concentration of carbon sources (particularly fructose in this study) is known to enhance lipid production in all oleaginous microorganisms including thraustochytrids as it provides excess continuous carbon flux in the central carbon backbone which then enters the lipid biosynthesis pathway upon nitrogen limitation is reached [28]. On the other hand, yeast extract provides essential vitamins particularly B complex which is vital for cell growth, thus directly correlates to its positive effects on the DHA volumetric content by positively influencing biomass concentration. Nevertheless, in contrast to the previous study, it was found that fructose-MSG interaction has a negative effect on DHA concentration [29]. Similarly, another study aimed at medium optimization for SW1 revealed that glucose, neither as a single factor nor in interaction, affect DHA concentration significantly [12]. This clearly shows that improvement of DHA accumulation by SW1 can better be achieved using fructose, compared to the most conventional substrate, glucose. Furthermore, a positively significant 3-factor interaction of fructose-MSG-sea salt (ACD) observed in the current study corresponds with the previous finding, where glucose-MSG interaction was found to positively affect DHA concentration [29].

While DHA biosynthetic capacity serves as a good indicator of the capability of the cells to synthesize DHA, it is not always a definitive indicator for DHA production. Cultivation that results in high biomass concentration but with low biosynthetic capacity can still result in more products compared to those with low biomass and high biosynthetic capacity. Therefore, to achieve optimal production, it is important to consider both volumetric DHA content together with biosynthetic capacity. Since fructose and its interaction with yeast extract (AB) were found to have a significant positive effect on both volumetric DHA content and DHA biosynthetic capacity, it can be concluded that manipulation of these two factors is crucial in any attempts to enhance the production.

Having a better preference for fructose compared to glucose for both biomass and DHA accumulation is less common among the known

Table 5. Examples of *thraustochytrids* and their fructose assimilation capabilities.

Strain	Fructose assimilative capability	Reference
S. aggregatum	×	[17]
T. aureum ATCC 34304	×	[18,19]
S. limacinum sp. nov.	✓	[20]
A. limacinum SR21	✓	[21]
S. limacinum BR2.1.2	✓*	[13]
S. mangeovei PQ6	✓	[22]
Aurantiochytrium sp. SD116	✓	[23]
Thraustochytrium sp. ONC-T18	✓	[24]
Aurantiochytrium sp. YLH70	✓*	[25]
Aurantiochytrium sp. SW1	✓*	[10]
Aurantiochytrium sp. CB15-5	✓	[26]
Aurantiochytrium sp. ZJWZ-7	✓*	[27]

* prefer fructose over glucose for biomass and DHA accumulation.

thraustochytrids. As listed in Table 3, SW1, *Aurantiochytrium* sp. YLH70 [25], *S.limacinum* BR2.1.2 [13] and *Aurantiochytrium* sp. ZJWZ-7 [27] are among the few reported strains that possess this unique preference. This unique attribute possibly due to a more active fructose transport system within the membrane of these Thraustochytrids, as what was observed in the fructose-preferred *Corynebacterium glutamicum* which showed that fructose was predominantly catabolized through phosphoenolpyruvate-dependent phosphotransferase systems, resulting in a major entry of fructose via fructose 1,6-bisphosphate for the production of dihydroxyacetone [30]. Similar mechanisms may be involved in the metabolism of fructose by SW1 and the other fructose-preferred thraustochytrids, but, further work needs to be conducted to confirm the claim. Besides, it could be also explained by reflecting the metabolism of the sugars in which the first reaction in fructose metabolism is the direct formation of fructose 1-phosphate by phosphoenolpyruvate (PEP) while the metabolism of glucose required an extra step in which glucose needs to be converted into glucose 6-phosphate first and then to fructose 6-phosphate by glucose-6-phosphate isomerase with the expense of one ATP molecule. Thus, utilizing fructose as carbon sources require less energy and steps in comparison to glucose, which may result in higher fructose in intake as compared to glucose [31,32]. This will then led to excess continuous carbon flux which enters the lipid biosynthesis pathway upon cessation of growth as a nitrogen-limited condition is reached, explaining the higher DHA production achieved in this study. However, no reports are available on further studies involving fructose as a major or supplemental carbon source. This can be related to the aspect of cost, where fructose is generally costlier compared to glucose. Since almost every study in the field of microbial DHA prioritizes economics of production, fructose is often not studied as a possible source of carbon. Nevertheless, further investigations on the fructose assimilative capabilities of some thraustochytrids could lead to new discoveries and possibilities in this field.

In conclusion, fructose serves as a positively significant carbon source for DHA accumulation by *Aurantiochytrium* sp. SW1 and its effects are more profound than that of glucose. Therefore, incorporation of fructose and/or fructose containing substrates in growth media could result in better DHA productivities.

Declarations

Author contribution statement

Vidyah Manikan: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Yusuf Nazir: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Aidil Abdul Hamid: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Funding statement

This work was supported by MINISTRY OF HIGHER EDUCATION MALAYSIA under the Fundamental Research Grant Scheme, grant number FRGS/2/2013/SG05/UKM/02/1.

Data availability statement

Data included in article/supp. material/referenced in article.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

[1] W.S. Harris, M.L. Baack, Beyond building better brains: bridging the docosahexaenoic acid (DHA) gap of prematurity, J. Perinatol. 35 (1) (2015) 1–7.
[2] S.J. Carlson, E.M. Fallon, B.T. Kalish, K.M. Gura, M. Puder, The role of the ω-3 fatty acid DHA in the human life cycle, J. Parenter. Enteral Nutr. 37 (1) (2013) 15–22.
[3] E. Peltomaa, M.D. Johnson, S.J. Taipale, Marine cryptophytes are great sources of EPA and DHA, Mar. Drugs 16 (1) (2018) 3.
[4] D.J. Kyle, V.J. Skotte, S.E. Reeb, Bioproduction of docosahexaenoic acid (DHA) by microalgae, in: D.J. Kyle, C. Ratledge (Eds.), Single Cell Oil, Am Oil Chem Soc, Champaign, 1992, pp. 287–320.
[5] O. Pulz, W. Gross, Valuable products from biotechnology of microalgae, Appl. Microbiol. Biotechnol. 65 (6) (2004) 635–648.
[6] V.J. Furlan, V. Maus, I. Batista, N.M. Bandarra, Production of docosahexaenoic acid by *Aurantiochytrium* sp. ATCC PRA-276, Braz. J. Microbiol. 48 (2) (2017) 359–365.
[7] C. Corinaldesi, G. Barone, F. Marcellini, A. Dell'Anno, R. Danovaro, Marine microbial-derived molecules and their potential use in cosmeceutical and cosmetic products, Mar. Drugs 15 (4) (2017) 118.
[8] E. Nutahara, E. Abe, S. Uno, Y. Ishibashi, T. Watanabe, M. Hayashi, N. Okino, M. Ito, The glycerol-3-phosphate acyltransferase PLAT2 functions in the generation of DHA-rich glycerolipids in *Aurantiochytrium limacinum* F26-b, PloS One 14 (1) (2019), e0211164.
[9] T.E. Lewis, P.D. Nichols, T.A. McMeekin, The biotechnological potential of thraustochytrids, Mar. Biotechnol. 1 (6) (1999) 580–587.
[10] V. Manikan, M.Y. Nazir, M.S. Kalil, M.H. Isa, A.J. Kader, W.M. Yusoff, A.A. Hamid, A new strain of docosahexaenoic acid producing microalga from Malaysian coastal waters, Algal Res 9 (2015) 40–47.
[11] M.J. Anderson, P.J. Whitcomb, DOE Simplified: Practical Tools for Effective Experimentation, second ed., Productivity Press, New York, 2007.
[12] V. Manikan, M.S. Kalil, A.A. Hamid, Response surface optimization of culture medium for enhanced docosahexaenoic acid production by a Malaysian thraustochytrid, Sci. Rep. 5 (2015) 8611.
[13] W. Chatdumrong, W. Yongmanitchai, S. Limtong, W. Worawattanamateekul, Optimization of docosahexaenoic acid (DHA) production and improvement of

astaxanthin content in a mutant *Schizochytrium limacinum* isolated from mangrove forest in Thailand, Nat. Sci. 41 (2007) 324–334.
[14] Y. Nazir, H. Halim, P. Prabhakaran, X. Ren, T. Naz, H. Mohamed, S. Nosheen, K. Mustafa, W. Yang, A. Abdul Hamid, Y. Song, Different classes of phytohormones act synergistically to enhance the growth, lipid and DHA biosynthetic capacity of Aurantiochytrium sp. SW1, Biomolecules 10 (5) (2020) 755.
[15] Y. Nazir, S. Shuib, M.S. Kalil, Y. Song, A.A. Hamid, Optimization of culture conditions for enhanced growth, lipid and docosahexaenoic acid (DHA) production of Aurantiochytrium SW1 by response surface methodology, Sci. Rep. 8 (1) (2018) 1–12.
[16] C. Morabito, C. Bournaud, C. Maës, M. Schuler, R.A. Cigliano, Y. Dellero, E. Maréchal, A. Amato, F. Rébeillé, The lipid metabolism in thraustochytrids, Prog. Lipid Res. 101007 (2019).
[17] G. Bahnweg, Studies on the physiology of the thraustochytriales. II. Carbon nutrition of thraustochytrium spp., Schizochytrium sp., japonochytrium sp., ulkenia spp. and labyrinthuloides spp, Veroff Inst Meeresforsch Bremerh 17 (1979) 269–273.
[18] A. Singh, S. Wilson, O.P. Ward, Docosahexaenoic acid (DHA) production by *Thraustochytrium* sp. ATCC 20892, World J. Microbiol. Biotechnol. 12 (1) (1996) 76–81.
[19] P.K. Bajpai, P. Bajpai, O.P. Ward, Optimization of production of docosahexaenoic acid (DHA) by *Thraustochytrium aureum* ATCC 34304, J. Am. Oil Chem. Soc. 68 (7) (1991) 509–514.
[20] D. Honda, T. Yokochi, T. Nakahara, M. Erata, T. Higashihara, *Schizochytrium limacinum* sp. nov., a new thraustochytrid from a mangrove area in the west Pacific Ocean, Mycol. Res. 102 (4) (1998) 439–448.
[21] T. Yokochi, D. Honda, T. Higashihara, T. Nakahara, Optimization of docosahexaenoic acid production by *Schizochytrium limacinum* SR21, Appl. Microbiol. Biotechnol. 49 (1) (1998) 72–76.
[22] D.D. Hong, H.T. Anh, N.T. Thu, Study on biological characteristics of heterotrophic marine microalga-*Schizochytrium mangrovei* PQ6 isolated from phu quoc island, Kien Giang province, Vietnam 1, J. Phycol. 47 (4) (2011) 944–954.
[23] M. Gao, X. Song, Y. Feng, W. Li, Q. Cui, Isolation and characterization of *Aurantiochytrium* species: high docosahexaenoic acid (DHA) production by the newly isolated microalga, *Aurantiochytrium* sp. SD116, J. Oleo Sci. 62 (3) (2013) 143–151.
[24] A.M. Burja, H. Radianingtyas, C.J. Barrow, A.J. Windust, U.S. Patent No. 8,921,069, U.S. Patent and Trademark Office, Washington, DC, 2014.
[25] X.J. Yu, Z.Q. Yu, Y.L. Liu, J. Sun, J.Y. Zheng, Z. Wang, Utilization of high-fructose corn syrup for biomass production containing high levels of docosahexaenoic acid by a newly isolated *Aurantiochytrium* sp. YLH70, Appl. Biochem. Biotechnol. 177 (6) (2015) 1229–1240.
[26] T. Tajima, K. Tomita, H. Miyahara, K. Watanabe, T. Aki, Y. Okamura, Y. Matsumura, Y. Nakashimada, J. Kato, Efficient conversion of mannitol derived from brown seaweed to fructose for fermentation with a thraustochytrid, J. Biosci. Bioeng. 125 (2) (2018) 180–184.
[27] Q. Wang, H. Ye, Y. Xie, Y. He, B. Sen, G. Wang, Culturable diversity and lipid production profile of labyrinthulomycete protists isolated from coastal mangrove habitats of China, Mar. Drugs 17 (5) (2019) 268.
[28] Y. Nazir, H. Halim, N.K.N. Al-Shorgani, V. Manikan, A.A. Hamid, Y. Song, Efficient conversion of extracts from low-cost, rejected fruits for high-valued Docosahexaenoic acid production by Aurantiochytrium sp. SW1, Algal Research 50 (2020) 101977, 2020.
[29] V. Manikan, M.S. Kalil, M.H. Isa, A.A. Hamid, Improved prediction for medium optimization using factorial screening for docosahexaenoic acid production by *Schizochytrium* SP. SW1, Am. J. Appl. Sci. 11 (3) (2014) 462–474.
[30] P. Kiefer, E. Heinzle, O. Zelder, C. Wittmann, Comparative metabolic flux analysis of lysine-producing Corynebacterium glutamicum cultured on glucose or fructose, Appl. Environ. Microbiol. 70 (1) (2004) 229–239.
[31] N.J. Patni, J.K. Alexander, Catabolism of fructose and mannitol in Clostridium thermocellum: presence of phosphoenolpyruvate: fructose phosphotransferase, fructose l-phosphate Kinase, phosphoenolpyruvate: mannitol phosphotransferase, and mannitol l-phosphate dehydrogenase in cell extracts, J. Bacteriol. 105 (1) (1971) 226–231.
[32] N.K.N. Al-Shorgani, M.H.M. Isa, W.M.W. Yusoff, M.S. Kalil, A.A. Hamid, Isolation of a Clostridium acetobutylicum strain and characterization of its fermentation performance on agricultural wastes, Renew. Energy 86 (2016) 459–465.