Journal of Industrial Microbiology & Biotechnology (1998) 20, 82–85

http://www.stockton-press.co.uk/jim

Spray-drying of *Dunaliella salina* to produce a β-carotene rich powder

G Leach, G Oliveira and R Morais

Laboratorio de Biotecnologia Vegetal-CBQF, Escola Superior de Biotecnologia-UCP, Rua Dr Antonio Bernardino de Almeida, 4200 Porto, Portugal

Powders of *Dunaliella salina* biomass were obtained by spray drying a cell concentrate under different drying regimes. A three-factor, two-level experimental design was employed to investigate the influence of inlet temperature, outlet temperature and feed solids on β-carotene recovery. The effect of microencapsulation in a polymer matrix of maltodextrin and gum arabic was also studied. All powders were stored under specific conditions to assess the stability of the native β-carotene. There was a trend indicating that lower outlet temperature yielded higher carotenoid recoveries, β-carotene recovery varying between 57% and 91%. Microencapsulated biomass yielded 100% recoveries. All non-microencapsulated powders were unstable in terms of β-carotene content in the presence of natural light and oxygen showing 90% degradation over a 7-day period. The incorporation of a microencapsulating agent had a significant increase in the storage stability. Results indicated a first-order degradation of the β-carotene in microencapsulated powders with kinetic constants of 0.06 day^{-1} and 0.10 day^{-1}. HPLC analysis showed no effect of drying processes on isomer composition (9-*cis*-β-carotene and *all-trans*-β-carotene ratio). This behaviour was also observed during storage of the microencapsulated powders.

Keywords: *Dunaliella salina*; microalgae; β-carotene; spray-drying; *cis/trans* isomers; microencapsulation

Introduction

Dunaliella salina is a motile unicellular alga belonging to the class Chlorophyceae. It can tolerate extreme variations in salinity ranging from 0.2–35% [4], but is most frequently found naturally in habitats with salinities above 10%, such as salt marshes and salinas. When exposed to a combination of high salinity, high UV radiation and low nitrate nutrient stress, *D. salina* massively accumulates β-carotene at levels that can reach 10% on a dry weight basis [2]—the highest levels found in nature. In addition to being a natural orange pigment, β-carotene is the most effective precursor of vitamin A, a potent antioxidant, and potentially, a cancer-preventing nutraceutical [7]. The *all-trans*-isomer of β-carotene has been chemically synthesised by Hoffmann La Roche since the 1950s; however, *cis* isomeric forms such as 9-*cis*, present in approximately equal proportions to *all-trans* in *D. salina* are significantly more lipid soluble and may thus accumulate more effectively in animal tissue [3]. In addition, a marketing advantage can be gained by labelling a product as 'natural'. Indeed natural β-carotene has a market price approximately double that of the synthetic product. A recent work stated that at least five companies were actively engaged in the industrial cultivation of *D. salina* for natural β-carotene [9], the halotolerance of the species permitting large-scale cultivation in outdoor raceway ponds or lagoons without fears of excessive predation or contamination.

Most commercially available products of natural β-carotene from *D. salina* are based on vegetable oil enriched with algal carotenoid [8]. An alternative product is a spray-dried biomass powder. β-carotene is very susceptible to oxidative degradation. Indeed, it has been reported that spray-drying using conventional air as the drying gas can result in 45% oxidation of carotenoids from an algal feed [6]. No work however has investigated the effect of different drying regimes on carotenoid recovery during spray-drying. Spray-dry microencapsulation has been employed to improve the stability of carotenoids in paprika oleoresin [1] and carrot pulp [10]; the latter work also investigated the effects of storage on the α and β structural isomers. In the case of *D. salina*, the relative stability of the *cis* and *trans* stereoisomers in the product is of key commercial importance. The objectives of this work are to investigate the effects of processing conditions on the β-carotene content and stereoisomer composition of spray-dried *D. salina* biomass and determine the effects of microencapsulation to improve the stability of the resultant powder.

Materials and methods

Experimental design

Three important drying parameters on the recovery of β-carotene during spray-drying of *D. salina* were investigated: the temperature of the drying air entering the dryer near the feed inlet (T inlet), the temperature of the air as it leaves the dryer (T outlet), and the solids content of the feed to the dryer (% solids w/w). A three-factor, two-level experimental design was consequently employed involving eight experimental runs. The effect of microencapsulation was not incorporated in this design, but investigated as two separate experiments in which only inlet temperature was varied. Each experiment was carried out twice.

Correspondence: R Morais, Laboratorio de Biotecnologia Vegetal-CBQF, Escola Superior de Biotecnologia-UCP, Rua Dr Antonio Bernardino de Almeida, 4200 Porto, Portugal
Received 16 October 1996; accepted 13 November 1997

D. salina culture

A volume of 125 L of live carotenised culture originating from Culture Collection of Algae and Protozoa, Cumbria, UK and subsequently grown in outdoor raceway ponds in the Algarve was supplied by Necton Lda (Belamandil, Algarve, Portugal). The culture had a cell concentration of 0.8×10^6 cells ml^{-1}, a salinity of 18% NaCl w/w, and a pH of 8.5. It was transferred to four 50-L clear polythene tubes located outside on the south side of the building in Porto and agitated by gentle sparging of compressed air to stabilise the culture and check its condition. Regular inputs of CO_2 and deionised water maintained the pH and salinity at their original values. Due to the extreme salinity, it was not necessary to ensure the sterility of all inputs. The culture was maintained in this condition with no noticeable decline in cell viability as observed by microscopy for 5 days. The culture was then concentrated and washed by low speed centrifugation at $2600 \times g$ to a cell concentration of 20×10^6 cells ml^{-1}. Feed solid levels were thus adjusted by desalting the culture using a centrifuge to salinities of 4% and 7% w/w, giving solids levels of 4.5% and 8.75% w/v respectively; salt was thus considered an inert bulking solid.

Spray-drying

Concentrated feed suspensions were spray dried using a Niro Atomiser Mobile Minor Unit (Niro Atomiser, Copenhagen, Denmark). The dryer had a chamber diameter of 0.8 m, and a rotating wheel atomiser which was operated at 23 000 rpm for all experiments. The inlet temperature was fixed by adjusting the heater controls on the dryer. Outlet temperature is controlled by the feed input rate to the dryer and was fixed by feeding deionised water prior to the introduction of the algal feed. The drying conditions used in the study are described in Table 1. During the drying experiments, the powder collection bottles were covered with aluminium foil to reduce the likelihood of post-drying photoxidation of the carotenoids during each experimental run. Only powder collected in the glass collection vessels was used for analysis and stability studies.

Microencapsulation

Microencapsulation of a more highly concentrated algal suspension (198×10^6 cells ml^{-1}) was carried out by mixing an equal volume of algal concentrate with a 22.5% w/v polymer mixture of maltodextrin dextrose equivalent 12 (Roquette, France) and gum arabic (Merck, Germany) combined in the ratio 3.5 : 1. The higher cell content was necessary to compensate for dilution by the polymer mix and also to partially compensate for the significant increase in solids levels causing a relative reduction in carotenoid as a percentage of solids.

Analysis

Feed to the spray-dryer and resultant powder were dried overnight at 105°C for total solids determination. Carotenoids were extracted in triplicate from the feed and biomass powders with acetone and determined spectrophotometrically at 450 nm in a standard volume of the same solvent using an extinction coefficient of 2500. Powder samples containing the microencapsulating mixture had to first be vigorously mixed and sonicated with deionised water prior to extraction with acetone. Acetone extracts were evaporated under nitrogen and redissolved in methanol/diethyl ether 4 : 1 and injected into a Beckman Gold HPLC system equipped with a diode array detector. Carotenoids were separated by a VYDAC 201 TP C18 reverse phase chromatography column (The Separations Group, Hesperia, CA, USA). The column temperature was maintained at 30°C. The binary gradient was composed of methanol (solvent A) and acetonitrile (solvent B). The course of the gradient was 100% A for 5 min, 90% A and 10% B at 5.1 min for 30 min, followed by 100% A at 35.1 min for 20 min. The flow rate was 0.7 ml min^{-1}. Carotenoids were identified by comparison of retention time with standards provided by Roche (Basel, Switzerland) and by analysis of the absorption spectra of the eluting peaks.

β-carotene stability study

After each drying, the glass vessels were allowed to equilibrate to room temperature. Subsequently, some of the powder was then transferred to 25-ml glass vials and exposed to daylight in the laboratory. The average light intensity to which the samples were exposed was 11.8 μmol photon m^{-2} s^{-1}. Samples were taken after day 2 and day 7 for carotenoid determination and thereafter weekly for a 36-day period.

Table 1 Effect of spray-drying conditions on β-carotene recoveries in *D. salina* biomass

Feed solids (% w/v)	T inlet (°C)	T outlet (°C)	Powder solids (% w/w)	β-carotene (% w/w solids)	β-carotene recovery (%)	β-carotene in starting material (% w/w)
4.50	200	110	95.85 ± 0.98	0.62 ± 0.02	90.89	0.68
		120	98.26 ± 1.32	0.79 ± 0.04	76.23	1.04
	265	110	95.28 ± 0.92	0.56 ± 0.05	81.12	0.69
		120	95.06 ± 0.30	0.47 ± 0.02	81.36	0.58
8.75	200	110	94.72 ± 0.46	0.24 ± 0.01	67.54	0.36
		120	94.55 ± 0.18	0.16 ± 0.02	56.92	0.28
	265	110	95.84 ± 0.54	0.27 ± 0.01	90.33	0.30
		120	94.61 ± 0.15	0.34 ± 0.02	82.93	0.40
15.43[a]	200	120	98.83 ± 0.17	0.21 ± 0.01	100.00	0.21
	265	120	98.15 ± 0.20	0.22 ± 0.01	100.00	0.22

[a]Microencapsulated.

Statistical analysis

Statistical analysis of the experimental design for significant effects and interactions of experimental factors was performed using Statgraphics statistical software (Statistical Graphics Corporation, Rockville, MD, USA).

Results and discussion

All the drying experiments yielded orange, dry, free-flowing powders. The most significant characteristic from an industrial point of view is the β-carotene recovery value for each condition (Table 1). As referred to in Material and Methods, the original culture was concentrated by centrifugation to obtain two concentrated suspensions (4% salt, 4.5% solids and 7% salt, 8.75% solids). Due to the practical impossibility of doing more than two drying experiments per day, the concentrated cultures were kept in the freezer for 7 days until being processed. This storage resulted in inevitable depletion of β-carotene. Before each assay, β-carotene and solids content of concentrated culture were determined. Results showed no variation in solids, but there was a decrease in β-carotene concentration with time. Values of β-carotene content of the concentrated culture (spray-dried feed) are represented in Table 1 for each experiment. It is clear, that contrary to the work of [6] with the microalgae *Spirulina* spp, conventional spray drying can be used to dry *D. salina* without excessive β-carotene degradation under appropriate conditions, two experiments yielding β-carotene recoveries in excess of 90%. At low feed solids levels, variations in inlet and outlet temperature are less important, the combination of low inlet and low outlet providing the best recovery of 90.89%. At higher solids levels that would be more typical of an industrial process, a higher inlet temperature was clearly more effective at reducing degradation. For all combinations of solids levels and inlet temperature, a low outlet temperature produced a better β-carotene recovery. No significant ($P<0.05$) interactions were found between the three experimental parameters studied (inlet temperature, outlet temperature, feed solids content).

Figure 1 compares the chromatograms of carotenoid extracted from the feed to the spray-dryer for one of the experiments and the resultant spray-dried powder. Both chromatograms clearly show a high amount of 9-*cis* isomer

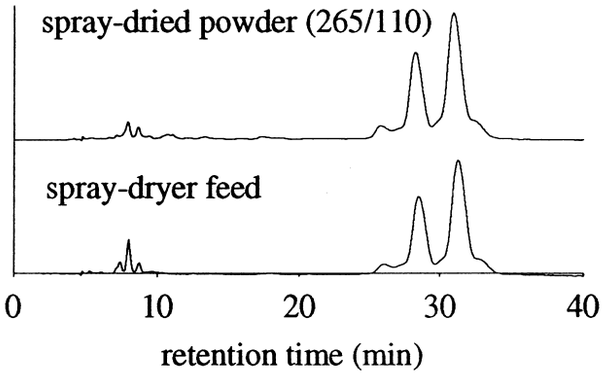

Figure 1 HPLC carotenoid profile at 450 nm showing the effect of spray-drying (conditions T inlet 265°C, T outlet 110°C, solids content of feed 4.5% w/w) on β-carotene isomers of *D. salina*.

typical for *D. salina* and are virtually identical, confirming that no discernible changes in isomer proportions resulted from the drying process. Despite the apparently high temperatures used in spray drying processes, the generation of a highly atomised spray provides a large surface area for the rapid evaporation of water. Consequently, the mean temperature of the drops is low enough, and the residence time in the drying chamber of the dried particles short enough, that excessive oxidation of carotenoid is avoided. In the case of runs with high outlet temperatures (low inlet feed rate), the 'real' temperature to which the biomass is exposed during the run is high, probably a consequence of the extremely high specific surface typical of spray-dried products, which maximises exposure of carotenoids to oxygen during storage [5]. The microencapsulated powders were considerably more stable due to the action of the polymer coat as a barrier to oxygen diffusion; consequently, sufficient data points were obtained to apply a first order kinetic model, which fitted both data sets well. First order degradation constants for microencapsulated powders are: 0.06 day^{-1} ($r^2 = 0.98$) for 200°C inlet temperature and 0.10 day^{-1} ($r^2 = 0.99$) for 265°C. Of particular note is that the powder produced with a lower inlet temperature was more stable. This is probably due to the effect of temperature on the nature of the polymer matrix during drying which is reflected in terms of its permeability to oxygen during storage. During the drying process, water must continue to evaporate through the drying matrix. At higher temperatures, the build-up of pressure and subsequent release of water vapour can lead to a more open voided structure where exposure to oxygen and thus degradation is greater. All non-encapsulated powders exhibited a rapid degradation to levels below 10% of the original carotene levels after only 7 days. On the contrary, in microencapsulated powders despite the fact that some degradation has occurred, the proportions of the isomers are virtually unaltered from those shown in Figure 1. This indicates that the degradative mechanism affects both isomers equally.

It can be concluded that by choice of appropriate drying conditions, most notably avoidance of high outlet temperatures, spray-drying can be used to produce a β-carotene rich powder from *D. salina*, without excessive degradation or change in isomer composition. The β-carotene in the resulting powders degrades rapidly in the presence of light and oxygen. A dramatic improvement in the β-carotene stability of the resultant powders can be obtained by spray-drying the cell concentrate with a microencapsulating polymer mix of maltodextrin and gum arabic. A similar result was also observed by [10] using hydrolyzed starches as encapsulating agent.

Preliminary studies with the addition of BHT, TBHQ and vitamin E to the spray dryer feed indicated that only TBHQ may be successful in significantly reducing degradation of the non-encapsulated powders during storage in the presence of light and oxygen.

For the spray drying of *Dunaliella* to be economically feasible, it will be necessary to further reduce salt concentration in the biomass. By careful step-wise centrifugation it is possible to reach 1–2% of salt without disrupting the cells, although this may not be feasible at industrial scale. Other methods of desalinization have been tried (dialysis

and ultrafiltration) but have not been put into practice due to inefficiency (dialysis) or damage to *D. salina* cells (ultrafiltration).

Acknowledgements

The authors would like to acknowledge the technical assistance given by Sandra Sa. Gareth Leach gratefully acknowledges support from an EU post-doctoral AAIR bursary. This work was supported by the research programme AIR2-CT94-1283.

References

1 Beatus Y, A Raziel, M Rosenberg and IJ Kopelman. 1985. Spray-drying microencapsulation of paprika oleoresin. Lebensm -Wiss u -Technol 18: 28–34.
2 Ben-Amotz A. 1993. Production of β-carotene and vitamins by the halotolerant alga *Dunaliella*. In: Marine Biotechnology Volume 1: Pharmaceutical and Bioactive Natural Products (Attaway DH and Zaborsky OR, eds), pp 411–417, Plenum Press, New York.
3 Ben-Amotz A, S Mokady, S Edelstein and M Avron. 1989. Bioavailability of natural isomer mixture as compared with synthetic all-trans β-carotene in rats and chicks. Am Inst Nutrition 22: 1013.
4 Borowitzka LJ. 1981. The microflora. Adaptations to life in extremely saline lakes. Hydrobiologia 81: 33–46.
5 Chou H and WM Breene. 1972. Oxidative decoloration of β-carotene in low-moisture model systems. J Food Sci 37: 66–68.
6 Cysewski GR. 1994. Ocean-chill drying of microalgal and microalgal products. USA Patent 5 276 977.
7 Germann I. 1994. Beta-carotene, inducer of cell–cell communication. Int Food Ingredients 4: 21–24.
8 Nonomura AM. 1987. Process for producing a naturally derived carotene-oil composition by direct extraction from algae. USA Patent 4 680 314.
9 Phillips TD. 1993. Stress manipulation in *Dunaliella salina* and dual-stage β-carotene production. PhD Thesis. University of Rhodes, South Africa.
10 Wagner LA and JJ Warthesen. 1995. Stability of spray-dried encapsulated carrot carotenes. J Food Sci 60: 1048–1053.