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Comparison between freeze and spray drying to obtain powder *Rubrivivax gelatinosus* biomass

Comparação entre a secagem por liofilização e atomização para produção de biomassa bacteriana

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Abstract

The use of colorants in products of animal origin is justified by the improvement in the color of foods since this attribute is considered a quality criterion. These additives can be produced using industrial effluents as substrates and appropriate organisms, such as *Rubrivivax gelatinosus*. Oxycarotenoids represent a class of carotenes responsible for the pigmentation of animals and vegetables. *R. gelatinosus* grows in fish industry effluent with the resulting production of a bacterial biomass containing oxycarotenoids. The purpose of this study was to compare the use of two drying processes - spray and freeze drying - to obtain powder biomass in terms of the process parameters (yield, productivity, and product recovery) and the product characteristics (color, proximate composition, and oxycarotenoids). No difference was detected in the yield between these techniques, while productivity was higher using spray drying. Higher product recovery and moisture were achieved with freeze drying, while ash was higher with spray drying. The freeze dried biomass was redder, darker and less saturated than the spray dried biomass. No difference in oxycarotenoids was detected between the biomasses. Although it results in lower recovery rate, spray drying was faster and more productive, and it provided the same yield as freeze drying, which makes it the method of choice for obtaining *R. gelatinosus* biomass.

Keywords: biomass; biological pigments; *Rubrivivax gelatinosus*.

Resumo

O uso de corantes em produtos de origem animal justifica-se pela melhora na cor dos alimentos, uma vez que este atributo é considerado um critério de qualidade. Estes aditivos podem ser produzidos utilizando efluentes industriais como substratos e organismos adequados, como *Rubrivivax gelatinosus*. Oxicarotenóides representam uma classe de carotenos, responsáveis pela coloração de animais e vegetais. *R. gelatinosus* cresce em efluente de indústria de pescado produzindo biomassa contendo oxicarotenóides. O objetivo deste experimento foi comparar duas metodologias para obter a biomassa seca - atomização e liofilização - em relação aos parâmetros do processo (rendimento, produtividade, recuperação) e sobre as características dos produtos (cor, composição centesimal, oxicarotenóides). As técnicas não diferiram quanto ao rendimento, enquanto que a produtividade foi maior para a atomização. A maior recuperação e a maior umidade foram obtidas pela liofilização, enquanto que a concentração de cinzas foi maior com a atomização. A biomassa liofilizada foi mais vermelha, mais escura e de cor menos saturada. Não houve diferença entre os conteúdos de oxicarotenóides. Embora resulte em uma menor recuperação, a atomização foi mais rápida, mais produtiva e apresentou rendimento equivalente à liofilização, o que a torna o método de escolha para a obtenção de biomassa de *R. gelatinosus*.

Palavras-chave: biomassa; pigmentos biológicos; *Rubrivivax gelatinosus*.

1 Introduction

Color is an important attribute in food acceptance. It can be a quality attribute since things are perceived based on sensory impressions (BARUFFALDI; OLIVEIRA, 1998). Carotenoids are the natural pigments responsible for yellow, orange, and red colors in foods, pharmaceuticals, cosmetics, and animal feed. They consist of two classes of molecules: carotenes, which are strictly hydrocarbons, and xanthophylls or oxycarotenoids, which contain oxygen (JOHNSON, 2007).

Besides being widely used as colorants, some carotenoids can also be used to enrich foods due to their antioxidant properties, to the fact that they act as vitamin A precursor are considered beneficial for health, among other things (NIIZU,

2003; VALDUGA et al., 2009). The use of colorants in animal feeds provides typical pigmentation improving their consumer acceptance (MOTTA; VIDAL; MATTOS, 2009). For instance, canthaxanthin and bixin may be added to poultry feed to enhance egg yolk color and to broiler chicken feed to enhance skin and meat color, thus aggregating commercial value to these products (GARCIA et al., 2009; CARNEIRO, 2010). β -caroten and astaxanthin are the main pigments used in aquaculture (AKSU; EREN, 2007). These synthetic pigments are generally used to provide color to salmonids and trouts to make them more attractive to consumers (TAKAHASHI; TSUKAMOTO; TABAT, 2008).

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In 1996, the expression Single Cell Protein (SCP) was coined to describe the protein production in the biomass of different microbial sources that could be obtained from many substrates, such as industrial by-products. The use of SCP in human or animal nutrition has positive aspects due to its typical high concentration of nutrients. Nevertheless, some negative aspects may also be attributed to SCP since its consumption predisposes to the development of gout and renal stones, in addition to the possibility of contamination with pathogenic and toxic substances (UYSAL; AYDOGAN; ALGUR, 2002; NASSERI et al., 2011).

The great amounts of effluents from animal food industries has led to investigations on the use of these by-products as substrates for the growth of phototrophic bacteria that consume organic matter and produce bacterial biomass (PONSANO; LACAVA; PINTO, 2003; AZAD et al., 2004; KANTACHOTE; TORPEE; UMSAKUL, 2005). Due to the nutritional composition of this biomass, some authors have suggested its use as a biofertilizer and animal feed supplement (PONSANO; LACAVA; PINTO, 2003; PONSANO et al., 2004; KANTACHOTE; TORPEE; UMSAKUL, 2005). Studies conducted with a particular phototrophic bacterium, *Rubrivivax gelatinosus*, in effluents from poultry and fish industries demonstrated this micro-organism ability to produce biomass that can be used as a nutritional and color additive in animal feed (PONSANO; LACAVA; PINTO, 2003; PONSANO et al., 2004; PONSANO; LIMA; TORRES, 2011; SANTO, 2011).

Drying of food and feed ingredients may be performed in different dryers, according to the initial properties and final desired characteristics of the product and economic factors (BARUFFALDI; OLIVEIRA, 1998). Freeze drying, a technique based on water removal by sublimation, is used to obtain several industrial products (ROSA; TSUKADA; FREITAS, 2006). During freeze drying, substances are not exposed to high temperatures; therefore the freeze-dried products preserve their initial nutritious characteristics, and return to their original shape and texture instantaneously achieving long shelf life (BARUFFALDI; OLIVEIRA, 1998; PEREDA et al., 2005). This technique was used by Ponsano et al. (2002); Ponsano, Paulino and Pinto (2008); Lima, Ponsano and Pinto (2011) and Santo (2011) to dry *R. gelatinosus* biomass produced in industrial wastewater. On the other hand, spray drying technique consists of maximizing heat transfer, and it can be used for any product with a liquid-like behavior (BARUFFALDI; OLIVEIRA, 1998). Due to its versatility and speed, spray drying became the most used drying technique for heat sensible substances such as foods and biological materials (LABMAQ..., 2010; ROSA; TSUKADA; FREITAS, 2006). During this process, water removal is very fast, the final quality of the products is excellent, texture is maintained, and rehydration is quick (BARUFFALDI; OLIVEIRA, 1998; FELLOWS, 2006). Since the bacterial culture for biomass production in industrial effluents has a liquid-like behavior, it is feasible that spray drying technique works well for removing water from this product; hence we decided to investigate that possibility.

Therefore, the aim of this research was to investigate the effects of using freeze and spray drying to obtain of *R. gelatinosus*

biomass on the process parameters (yield, product recovery, and productivity) and on the product characteristics (composition, oxycarotenoids, and color).

2 Materials and methods

2.1 Microorganism, cell culture reactivation, pre-inoculum, and inoculum

Rubrivivax gelatinosus isolated from poultry slaughterhouse wastewater and characterized by morphological and biochemical tests was used in this experiment (PONSANO; LACAVA; PINTO, 2002). The cells were maintained in Pfennig semi-solid agar, and cell culture reactivation was performed according to the procedures previously described by Ponsano et al. (2002).

For the initial inoculum preparation, cells were grown in Pfennig liquid medium under anaerobiosis (fully filled screw-crap tubes), 32 ± 2 °C and $1,400 \pm 200$ lux for approximately 3 days until a slight red color developed. For the final inoculum, an aliquot from initial inoculum was transferred at 1% (v/v) to the same medium, and incubation was carried out in glass cylinders under the same conditions described before until optical density at 600 nm reached 0.5 (PONSANO; LACAVA; PINTO, 2003).

2.2 Substrate preparation

Tilapia fish processing wastewater used in this experiment was donated by Tilapia do Brasil Inc. (Buritama City, SP, Brazil) and was made up of effluents from killing, scaling, gutting, cleaning, skinning, filleting and freezing, and cleaning operations; it underwent treatment (grating) and was collected. In the laboratory, this effluent was filtered in 50 µm mesh fast filter (Gardena 1731; $3,000 \text{ L h}^{-1}$) for the removal of gross particles, and it was heat treated (Incomar LTLT tank) at 65 °C/30 min. After that, the wastewater was cooled to room temperature to receive the bacterial inoculum.

2.3 Production and drying of biomass

The bacterial inoculum was added to the treated wastewater at 1% (v/v), and anaerobic cultivation was carried out in 100 L glass reactors at 32 ± 2 °C and $2,000 \pm 500$ lux for seven days. After that time, the liquid bacterial cultures exhibited purple-red color, peculiar to *R. gelatinosus* growth in that substrate. Six bacterial cultivations were performed. The culture was filtered at 0.2 µm, $1.5 \text{ m}^3 \text{ h}^{-1}$ and 4.5 bar (Frings), resulting in a concentrate containing the cell mass. This concentrate was divided into two portions that were submitted to the two drying techniques. For the freeze drying process, the concentrate was initially centrifuged at 3,400 g for 30 minutes at 5 °C (Incibras Spin VI), and the resulting slime was frozen at -40 °C and freeze dried under vacuum at 140 L min^{-1} for 48 hours (Liobras L 101). Hand grinding was performed to obtain the powder freeze dried biomass. For the spray drying process, the concentrate was fed directly into a spray dryer (Labmaq MSDi 1,0) at entrance temperature of 120 °C, exit temperature of 65 °C, compressed air at 35 L min^{-1} , and peristaltic pump at 1 L h^{-1} to produce the powder spray dried biomass. Six production processes (freeze and spray drying processes) were performed to obtain the powder biomass.

2.4 Yield, recovery, and productivity

The yields of the drying processes were determined from the ratio between the solid matter of the final products and solid matter of the concentrate in the concentrate. Total solids in the biomasses were determined in an oven at 105 °C until constant weight. For the concentrate, the total solids were determined by taking 100 mL, which were initially evaporated in a water bath at 100 °C, and then dried to constant weight in an oven at 105 °C. Biomass recovery was determined based on the weight of the final products obtained by the two techniques. Productivity was the ratio between the final amount of product formed and drying process time.

2.5 Chemical composition

To determine the proximate composition of the biomasses, the following determinations were performed in duplicate: moisture at 105 °C, lipids (extraction in ethyl ether), Kjeldahl N for proteins, and ash at 550 °C (INSTITUTO..., 2008).

2.6 Color

Objective color was determined in the *LCh* space, in which the color attributes are *L* (*Lightness*), quality that differentiates a dark color from a light one; *C* (*Chroma*), that denotes the color saturation; and *h* (*hue*), which defines the color itself by an angular measurement: 0° (red), 90° (yellow), 180° (green) and 270° (blue) (HUNTERLAB, 2010). Measurements (illuminant D65 and 2° observer) were obtained from the average of three consecutive pulses launched on the biomasses from the optical chamber of the MiniScan XE Plus (Hunter Lab) colorimeter. Previous calibration was performed with black and white tiles.

2.7 Oxycarotenoids content

For the determination of oxycarotenoids, an adaptation of Valduga (2005) methodology was used (duplicates for each sample). The pigments were extracted from the biomass with dimetilsulfoxide at 55 °C/30 min and alternated cycles of ultrasound at 40 kHz (Unique/USC 1800A) under shaking (Phoenix/P-56). Next, a mixture containing acetone:methanol (7:3, v/v) was added, the tubes were centrifuged at 3,400 g and 5 °C/10 min, and the supernatant was transferred to a 50 mL volumetric flask. Successive extractions were performed until no color remained in the cells or solvent. Final dilutions were made up with methanol, and the quantification of oxycarotenoids was accomplished at 448 nm (Hitachi U-1000/U-1100). Total carotenoids were estimated according to Davies (1976) using the absorption coefficient of carotenoids suggested by Liaen-Jensen and Jensen (1971).

2.8 Statistical analysis

The means were compared using the t-test and the GraphPad InStat Version 3.06 (VIEIRA, 1999); the significance level adopted was 5%.

3 Results and discussion

The data shown in Table 1 are the average yield, product recovery, and productivity. Although the yields produced using

both drying techniques were considered statistically equal ($p = 0.3634$), the amount of biomass recovered after spray drying was lower ($p = 0.004$), and productivity was higher ($p < 0.0001$) for this technique.

Although adhesiveness was not measured in this study, it may have been responsible for the lower product recovery for spray drying. Some foods and pharmaceuticals tend to adhere to the spray dryer chamber causing considerable losses during the drying process (BHANDARI; HOWES, 1999). On the other hand, this was not a problem for freeze drying. With regard to productivity, freeze drying required 48 hours for the water removal, but spray drying, under the experimental conditions used in this study, was accomplished within 2 hours; which explains the higher values of productivity found for this technique.

The low product recovery found for both drying techniques in this study can be justified by the growth conditions used since under anaerobic light conditions, are more likely to produce pigments than cells (PRASERTSAN; CHOORIT; SUWANNO, 1993).

Moisture contents were 98.71% for the concentrate obtained by membrane filtration, 3.65% for the spray dried biomass, and 4.15% for the freeze dried biomass. The mean proximate compositions for both products are shown in Table 2 (wet basis). The high concentration of proteins found in those products is a characteristic of phototrophic bacteria biomass, which indicates a probable use in animal feed (PONSANO; PINTO; GARCIA-NETO, 2003). Moisture content was higher for the freeze dried biomass ($p = 0.0022$), while ash was higher for the spray dried biomass ($p < 0.0001$). Proteins and lipids did not differ significantly between the products ($p = 0.355$ and $p = 0.3022$, respectively). The lower moisture content found for the spray dried biomass shows that this technique was more effective at removing water, which may be explained by the intense contact

Table 1. Drying parameters of *Rubrivivax gelatinosus* biomasses obtained using spray and freeze drying¹ techniques.

Parameter	Spray drying	Freeze drying
Yield (%)	74.53 ± 1.81 ^a	74.92 ± 1.77 ^a
Recovery (g)	12.32 ± 0.98 ^a	16.86 ± 0.48 ^b
Productivity (mg h ⁻¹)	6.17 ± 0.72 ^a	0.351 ± 0.011 ^b

¹Mean values and standard deviations. ^{ab}Means in the same row with different superscripts differ significantly ($p < 0.05$) by the t-test.

Table 2. Proximate composition (wet basis) of *Rubrivivax gelatinosus* biomasses obtained using spray and freeze drying¹ techniques.

Component (%)	Spray drying	Freeze drying
Moisture	3.65 ± 0.2 ^a	4.15 ± 0.19 ^b
Ash	6.65 ± 0.32 ^a	4.33 ± 0.41 ^b
Crude protein	54.24 ± 2.19 ^a	55.41 ± 1.92 ^a
Lipid	12.48 ± 1.03 ^a	11.72 ± 1.37 ^a
Non-nitrogen fraction	22.98 ± 1.43 ^a	24.39 ± 1.55 ^a

¹Mean values and standard deviations. ^{ab}Means in the same row with different superscripts differ significantly ($p < 0.05$) by the t-test.

between the particles and the hot air (ROSA; TSUKADA; FREITAS, 2006). This was considered an important finding of this study, taking into account the association between water content and the stability of the biomass.

Some other authors investigated the proximate composition of *R. gelatinosus*, and they all agreed that it varies according to the substrate used for production as well as to its organic matter content. Ponsano, Lima and Torres (2011) used tilapia fish industry effluent as the substrate for the cultivation of the bacterium and found 57.39% protein, 11.08% lipids, 4.55% moisture, and 4.05% ash for the freeze dried biomass. These results are similar to those of this study. Ponsano, Lacava and Pinto (2003) used poultry slaughterhouse effluent as the substrate for the cultivation of the same bacterium and produced a freeze dried biomass containing 62.8% protein, 0.5% lipids, 7.1% moisture, and 4% ash.

All color attributes presented in Table 3 were higher for the spray dried product ($p < 0.0001$), demonstrating that this biomass is clearer, less red, and it has a more saturated color as compared to the freeze dried product. Such a difference must probably be due to the higher dynamics of the spray drying process, in which temperature, pressure, and particle size may change the color of the final product (FELLOWS, 2006; LABMAQ..., 2010). Moreover, another factor that may explain the differences in the color attributes is the different water content of the biomasses since drying processes modify the surface of a product altering its reflectiveness and color (FELLOWS, 2006).

The average contents of oxycarotenoids in the biomass were 3.74 and 3.72 mg g⁻¹ (dry basis) for the spray and freeze drying processes, respectively; these values were statistically the same ($p = 0.9293$). These values are also similar to those found by Prasertsan, Jaturapornpipat and Siripatana (1997) for *Rhodocyclus gelatinosus* R7 grown in tuna fish industry wastewater (from 2.13 to 3.90 mg g⁻¹). With respect to color, the determination of the carotenoids profile by High Performance Liquid Chromatography (HPLC) could explain the difference in color between the two processes evaluated.

In order to obtain good results with spray drying methodology, it is necessary to understand the physicochemical aspects of the materials involved as well as their interaction with the physical variables of the process (ROSA; TSUKADA; FREITAS, 2006; LABMAQ ..., 2010). The results presented in this study are preliminary data and, as soon as new studies are conducted, we intend to provide more data to make this

a more profitable technique to obtain *R. gelatinosus* biomass. For instance, the use of additives such as modified starch, maltodextrin, and gum Arabic may be tested to reduce losses caused by adhesion and to improve product recovery (WANG; LANGRISH, 2009; LABMAQ ..., 2010). Further studies will also be accomplished in order to investigate the economic feasibility of using this product in animal feeding.

4 Conclusions

The comparison between spray and freeze drying techniques to obtain powder *R. gelatinosus* biomass showed that the products have the same protein and lipid contents, but the lowest moisture was found using the spray dried technique, which means better preservation. Despite some differences found in the color of the products, they had the same oxycarotenoids contents. Although product recovery was lower for the spray drying technique, this technique was faster, more productive and provided the same yield as freeze drying, which makes it the method of choice for obtaining of *R. gelatinosus* biomass.

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Table 3. Color attributes (*L* - Lightness, *C* - Chroma, *h* - hue) of *Rubrivivax gelatinosus* biomasses obtained using spray and freeze drying¹ techniques.

Color attribute	Spray drying	Freeze drying
<i>L</i>	38.07 ± 2.14 ^a	19.68 ± 4.67 ^b
<i>C</i>	23.85 ± 0.84 ^a	10.09 ± 1.92 ^b
<i>h</i>	26.63 ± 1.84 ^a	20.01 ± 0.94 ^b

¹ Mean values and standard deviations. ^{ab} Means in the same row with different superscripts differ significantly ($p < 0.05$) by the t-test.

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