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Santos SOUZA, Alexandre; ROCHA-LEÃO, Maria Helena; Vilela BORGES, Soraia; CIRILLO, Marcelo Ângelo; Prado CORNEJO, Félix Emílio; COURI, Sônia
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Original

Retention of short chain fatty acids under drying and storage conditions

Retençao de ácidos graxos de cadeia curta sob secagem e condições de estocagem

Alexandre Santos SOUZA[1], Maria Helena ROCHA-LEÃO[2], Soraia Vilela BORGES[3*], Marcelo Ângelo CIRILLO[3], Félix Emílio Prado CORNEJO[4] , Sônia COURI[4]

Abstract

Cheese whey permeate was used as a substrate for the fermentation of *Propionibacterium freudenreichi* PS1 for the production of short chain fatty acids, components of the bio-aroma of Swiss cheese. The liquid bio-aroma was encapsulated by spray drying under different conditions of air inlet temperature and feed rate. A study was carried out on the stability of the bio-aroma during storage in laminated packages at 30 °C for 96 days using the product showing the greatest retention of acetic and propionic acids. The results showed that the best drying conditions were an air entrance temperature of 180 °C and a feed rate of 24 g/min resulting in particles with a smooth surface and few invaginations and micro-fissures. However, 72% of the acetic acid and 80% of the propionic acid were lost during storage showing that the wall material used was inadequate to guarantee product stability.

Keywords**:** *fermentation; microencapsulation; spray drying; Swiss cheese flavor*

Resumo

Permeado de soro de queijo foi usado como um substrato para a fermentação de Propionibacterium freudenreichi PS1 para produção de ácidos graxos de cadeia curta, constituintes do bioaroma de queijo suíço. O bioaroma líquido foi encapsulado por secagem por atomização sob diferentes condições de temperatura de entrada de ar e vazão de alimentação. Um estudo foi realizado sobre a estabilidade do bioaroma durante estocagem em embalagens laminadas a 30 °C por 90 dias, usando o produto que mostrou maior retenção de ácido acético e propiônico. Os resultados mostraram que a melhores condições de secagem foram uma temperatura do ar de entrada de 180 °C e taxa de alimentação de 24 g/min, resultando em partículas com superfície lisa e poucas invaginações e microfissuras. No entanto, 72% do ácido acético e 80% do ácido propiônico foram perdidos durante a estocagem, mostrando que o material de parede usado foi inadequado para garantir a estabilidade do produto.

Palavras-chave: *fermentação; microencapsulação; secagem por atomização; aroma de queijo suíço.*

1 Introduction

Whey is a byproduct of the cheese industry and is one of the most polluting residues produced by the food industry. Since it is rich in proteins and lactose, some alternatives have been examined by the dairy industry to find a use for it including ultrafiltration. Ultrafiltration of cheese whey separates the proteins retained in the concentrate, from lactose and permeate salt, and it can be used as a fermentation medium since in addition to its considerable lactose content, it also contains minerals and vitamins. Various studies have been carried out aimed at verifying the use of cheese whey and its permeate as substrates for fermentation (BRONSTEIN; ALEGRE, 1998). These include a study carried out by Teixeira et al. (2004) on the production of natural aromas using immobilized *Propionibacterium freudenreichii* cells for the production of the short chain fatty acids responsible for the aroma of Swiss cheese. Due to the volatile, unstable nature of these substances, the authors studied the encapsulation of the product by spray drying using different wall materials. However they did not study the effect of the drying and storage conditions on aroma retention.

Spray drying is one the most important encapsulation methods since it involves very rapid contact between the drops and hot air decreasing heat damage as well as showing high production capacity, amongst other advantages. It has therefore been applied in the microencapsulation of many volatile substances (ROSENBERG; KOPELMAN; TALMON,1990; BHANDARI et al., 1992; BUFFO; REINECCIUS, 2000; LIU et al., 2001; McNAMEE; O'RIORDAN; O'SULLIVAN, 2001; FINNEY; BUFFO; REINECCIUS, 2002; SOOTTITANTAWAT et al., 2003, 2005, TURCHIULI et al., 2006; BARANAUSKIENÉ et al., 2006) in addition to many other ingredients described in a recent up-dated literature survey on the subject by Gharsallaoui et al. (2007).

The retention of aroma components in microencapsuled products is important in the flavour perception of food

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[1] *Escola Agrotécnica Federal de Salina - EAFSALINAS, Salinas - MG, Brasil*
[2] *Departamento de Engenharia Bioquímica, Escola de Química, Universidade Federal do Rio de Janeiro - UFRJ, Rio de Janeiro, RJ, Brasil*
[3] *Departamento de Ciência dos Alimentos, Universidade Federal de Lavras - UFL, Campus Universitário, CEP 37200-000, Lavras, MG, Brasil e-mail: sborges@ufla.br*
[4] *EMBRAPA Agroindústria de Alimentos, Rio de Janeiro, RJ, Brasil*
**Corresponding author*

products since specific interactions result in bonding of the aroma components and food, such as by covalent bonding, hydrogen bridges, and hydrophobic interactions. Such retention is affected by drying process conditions, composition of the emulsion, nature of the wall materials, and encapsulated compounds. Amongst these, the increase in temperature of the air inlet accelerates the formation of a protective crust around the nucleus containing the volatile substance, preventing it from evaporating along with the water. However, excessively high temperatures cause expansion of the water vapor and the formation of structures that are externally wrinkled and internally porous, a phenomenon known as ballooning, which leads to a loss of aromas (RULKENS; THIJSSEN, 1967; ROSENBERG; KOPELMAN; TALMON, 1990; FINNEY; BUFFO; REINECCIUS, 2002; SOOTTITANTAWAT et al., 2003).

Few studies have been reported concerning the influence of the feed flow rate on the retention of volatiles since this variable is controlled by the outlet temperature, amongst other variables. The outlet temperature affects the moisture content of the product and has a similar effect to that of the air inlet temperature (BHANDARI et al., 1992).

The chemical and physical stability of dehydrated products depends on various factors, including the package material, storage temperature, and storage time (CANO-CHAUCA et al., 2005; ENDO et al., 2007; KANAKDANDE; BHOSALE; SINGHAL, 2007).

The objective of the present study was to verify the influence of spray drying conditions (air inlet temperature and feed rate) and storage conditions on the retention of short chain fatty acids produced by fermentation of the permeate of cheese whey by *Propionibacterium freudenreichii* PS-1.

2. Materials and methods

2.1 Materials

Cheese whey powder packaged in polyethylene bags (Alibra Ingredientes Ltda, Campinas, SP, Brazil), Calcium carbonate (Nuclear) was used as an anti-caking agent, and maltodextrin (Mor-rex 1910, Corn Products , Brazil) was used as the wall material in the preparation of the solution used in the microencapsulation process. The dried short chain fatty acids (or bio-aroma of Swiss cheese) were packed into metalized polyethylene bags provided by ITAP and constituted as follows: 12 μ*m* PET metalized/65 μ*m*, with oxygen permeability rates of 1.3-2.2 cm^3/m^2 day at 23 °C/100% RH, and water vapor permeability rates of 0.7-1.0 g/m^2 day at 36 °C/90% RH.

2.2 Methods

Ultrafiltration of the cheese whey

Following the manufacturer's instructions, the powdered cheese whey was reconstituted using 16 L of water per kg of powder in order to return to the *in natura* state of the cheese whey. About 40 L of reconstituted whey were ultrafiltered in a pilot ultrafiltration unit (Protosep IV, Koch Membrane Systems, WGM Sistemas, São Paulo, Brazil) with circulation using a centrifugal pump. A polysulfone membrane with a filtration area of 7.4 m^2 and cut-off point of 10 kDa was used. The operational conditions of the ultrafiltration system were as follows: the unit was programmed to use a temperature of 35 °C, inlet pressure of 3.5 bar, outlet pressure of 2.8 bar, and feed rate of 5.3 l/h^{-1}. After filtration, 39 L of permeate were filled into plastic containers (previously washed with neutral detergent and sanitized with a sodium hypochlorite solution at 200 $mg.kg^{-1}$, pH 10 for a residence time of 20 minutes, followed by rinsing with water to remove residual chlorine) and immediately frozen in a cold chamber at -16 °C until they were used for the fermentative process.

Preparation of the inoculum

The inoculum used in the fermentation process was prepared from 1 L of cheese whey permeate with the pH adjusted to 7 and autoclaved at 121 °C for 20 minutes in a vertical autoclave (Fabbe-Primar model 103, Itu, São Paulo, Brazil).

The permeate was then cooled to 30 °C and the culture of *Propionibacterium freudenreichi* PS1 from Christian Hansen added (10^{12} $UFC.ml^{-1}$) followed by incubation in a flask in an incubator maintained at 30 °C for 72 hours without agitation.

Fermentation of the permeate

Thirty litres of cheese whey permeate were used, with the pH adjusted to 7 using 0.2N NaOH, and divided between three vats (each with a volume of 14 L) for autoclaving. After autoclaving, the vats were allowed to cool naturally to 30 °C and 300 mL of inoculum poured into each vat followed by homogenization. The vats were then fitted into the bioreactor (Model FS 314, New Brunswick Scientific, INC, USA), where they remained for 96 hours to produce the organic acids. For the best production of organic acids during the fermentative process in the bioreactor, the temperature was maintained at 30 °C with no agitation or bubbling in of oxygen.

Feed preparation

For each trial, a solution was prepared using the proportion by weigtht: 1/10/100 (calcium carbonate (CC)/maltodextrin (MD)/fermented permeate) according to the results obtained previously by Teixeira et al. (2004). The calcium carbonate and maltodextrin were mixed with the fermented permeate resulting in a suspension with 16 °Brix.

Microencapsulation

Microencapsulation was carried out by drying the suspension in a mini spray dryer, model 190 Buchi (Buchi Laboratorius Technik, Flawil, Switzerland) operating under the various experimental conditions according to the 2^2 factorial experimental design with three central points using the methodology proposed by Box and Draper (1987). The factors evaluated were the air inlet temperature (T °C) and feed rate (Q g/min) as shown in Table 1, maintaining the maltodextrin and calcium carbonate concentrations constant resulting in a total of 7 trials. The air outlet temperature varied from 85

to 90 °C. After encapsulation, the product was immediately vacuum-packed in laminated packs using the SELOVAC model 200S vacuum packer (SELOVAC, São Paulo, Brazil). The models were adjusted using multi-linear regression with the aid of the Rsreg Sas Statistical Package (2000).

Characterization of the microcapsule structure

A JEOL model JSM 5310 scanning electronic microscope (SEM) was used for characterization of the microcapsule structure. The samples were placed on aluminum SEM stubs using double-sided adhesive carbon tape covered with a fine layer of gold using a coating unit (Balzers Union, model FL 9496) and analyzed using the SEM.

Fatty acids determination

The sample (1g) was dissolved in 10 mL distilled water and injected into a Waters 510 HPLC system equipped with a cation exchange column 8.0 × 300 mm (SHODEX Rspak KC-811) using isocratic elution with 0.6 M perchlorate at 0.6 mL/min and a column temperature of 70 °C.

Acetic and propionic acid retention

Equation 1 was used to evaluate the retention of acetic and propionic acids in the capsules obtained by spray drying:

$$R = \frac{AC(mg.g^{-1})}{AF(mg.g^{-1})} \times 100 \quad (1)$$

where:

AC = concentration of the acids in the capsules (dry weight basis);

AF = concentration of the acids in the fermented product (dry weight basis).

Study of the stability of the microencapsulated fatty acids

One gram of the samples from trial 2 (the trial resulting in greater retention of the fatty acids) was weighed in duplicate and vacuum-packed into laminated packs. The samples were maintained at 30 °C in an incubator for 96 days. They were then removed at five pre-determined intervals (0, 24, 48, 72, and 96 days) and analyzed for their fatty acid contents.

Table 1. Specifications for the experimental conditions of the factorial design.

Trial	x_1	T (°C)	x_2	Q (g/min)
1	+1	200	+1	24
2	-1	180	+1	24
3	+1	200	-1	16
4	-1	180	-1	16
5	0	190	0	20
6	0	190	0	20
7	0	190	0	20

x_1: coded variable for the drying air inlet temperature. x_2: coded variable for the feed flow rate.

3 Results and discussion

3.1 Retention of short chain fatty acids

Equations 2 and 3 describe the model obtained for the retention of acetic and propionic acids, respectively, presenting R^2/F regression values of 0.86/101.64 and 0.92/144.11, respectively, indicating a good fit to the experimental data.

$$\text{Acetic acid} = 33.62 - 4.38\ x_1 + 3.28\ x_2 - 3.58\ x_1x_2 \quad (2)$$

$$\text{Propionic acid} = 39.30 - 4.94\ x_1 + 5.05\ x_2 - 5.34\ x_1x_2 \quad (3)$$

where x_1 is coded variable for the drying air inlet temperature and x_2 is coded variable for the feed flow rate.

These relationships can be better visualized in Figures 1 and 2.

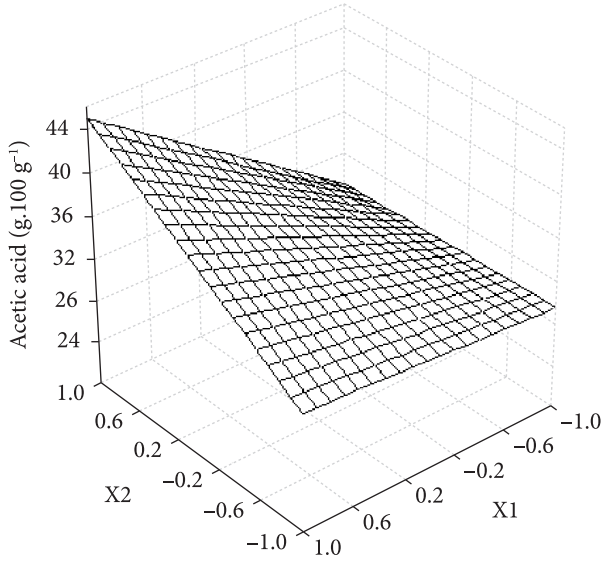

Figure 1. Response surface for the retention of acetic acid.

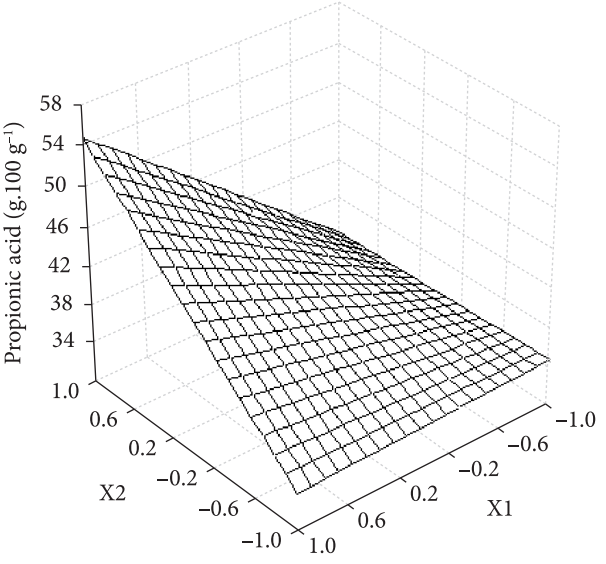

Figure 2. Response surface for the retention of propionic acid.

It can be seen that the values for retention were in the range from 30.24 to 47.37 g.100 g^{-1} microcapsule for acetic acid and from 35.98 to 57.12 g.100 g^{-1} microcapsule for propionic acid. Using immobilization of the microorganisms in alginate in the production of the same aromatic compounds, and subsequent spray drying at 180 °C (air inlet temperature) with a flow rate of 17 mL/min and maltodextrin as the coating material, Teixeira et al. (2004) obtained retentions of 33.78 g.100 g^{-1} microcapsule for acetic acid and 47.0 g.100 g^{-1} microcapsule for propionic acid, values smaller than those found in the present study.

An inverse relationship between the air inlet temperature and the feed rate can be seen in both figures with respect to the retention of short chain fatty acids. The retention increased with the increase in feed rate but decreased with the increase in temperature.

With respect to the temperature, some results published in the literature (ROSENBERG; KOPELMAN; TALMON, 1990, BHANDARI et al., 1992; FINNEY; BUFFO; REINECCIUS, 2002) registered an increase in retention with the increase in air inlet temperature due to the more rapid formation of a crust, but results similar to those found in the present research were found by Bhandari et al. (1992) due to the ballooning effect that occurred at high temperatures allowing for the escape of volatile components.

An increase in feed rate for the same inlet temperature resulted in a lowering of the outlet temperature (MAURY et al., 2005; TONON; BRABERT; HUBINGER,2008), which minimizes the loss of volatiles in the final stages of drying (RULKENS; THIJSSEN, 1967; ROSENBERG; KOPELMAN; TALMON, 1990).

3.2 Morphology of the microcapsules

Figure 3 shows the micrograph of the microcapsules obtained by the microencapsulation process in trial 2.

The morphology of the surface presents a smooth surface with few invaginations and total lack of micro-fissures, indicating that the encapsulation process of the acetic and propionic acids was highly successful, corroborating other results published in the literature (ROSENBERG; KOPELMAN; TALMON, 1990; ASCHIERI; MARQUEZ; MARTUCCI, 2003; TEIXEIRA et al. 2004; ELVERSSON; MILLQVIST-FUREBY, 2005; KANAKDANDE; BHOSALE; SINGHAL, 2007). This is due to rapid solidification of the microcapsule walls associated with the effects of higher drying temperatures amongst other variables, which, as mentioned earlier, minimizes the invaginations.

3.3 Stability of the short chain fatty acids

The stabilities of the acetic and propionic acids were investigated for the products resulting from trial 2, which presented the greatest retention of these compounds, and the results can be seen in Figure 4.

The results showed that the maltodextrin used as the wall material was inadequate for good retention of the acids, and the samples presented substantial losses of the volatiles in the first 48 days, up to 72% of the initial concentration for acetic and up to 80% for propionic acid. After the first 48 days, the losses remained constant reaching 82% for acetic acid at the end of the 96 days and 80% for the propionic acid. This slight difference in retention was due to the greater volatility of the acetic acid in relation to the propionic acid (GREEN; PERRY, 2007) due to its lower molecular weight. Such losses may be due to destruction of the microcapsule due to moisture absorption or internal porosity that allows the migration of volatiles during storage (ROSENBERG; KOPELMAN; TALMON, 1990).

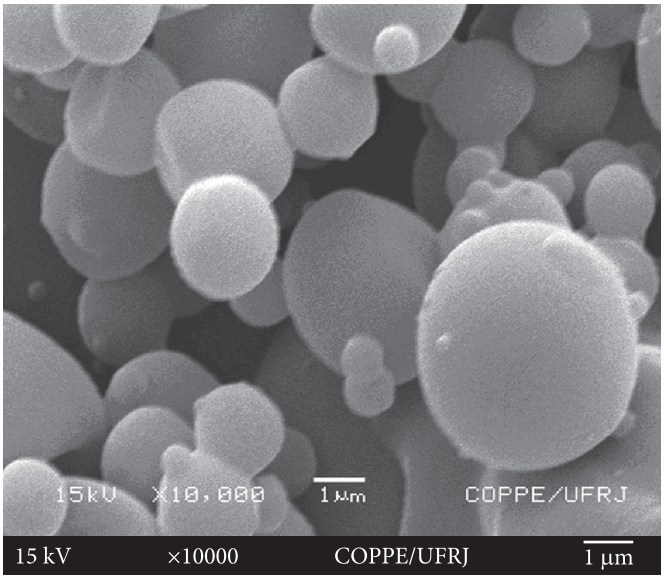

Figure 3. Morphology of the microcapsule.

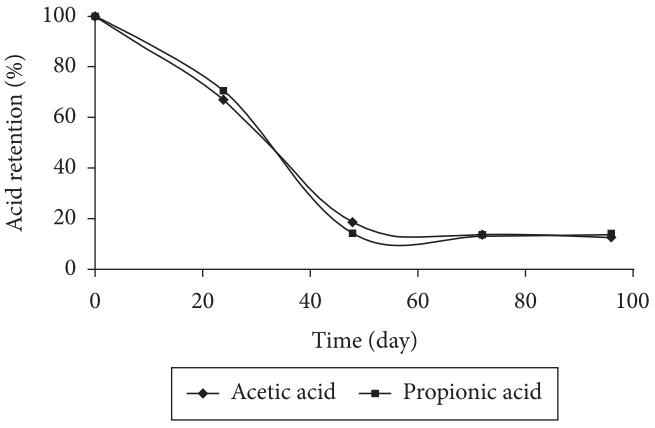

Figure 4. Alterations in the concentrations of acetic and propionic acids during storage.

4 Conclusions

Retention of both the acetic and propionic acids increased with the increase in feed rate and decreased with the increase in air inlet temperature. The best drying conditions for greater retention of both acids were the use of an air inlet temperature of 180 °C and feed rate of 24 g/min, which resulted in the formation of smooth surfaced microcapsules showing few invaginations and no micro-fissures. The maltodextrin used as the wall material was inadequate for good retention of the short chain fatty acids (or bio-aroma) showing losses greater then 80 g.100 g^{-1} in about three months of storage in a package considered as a good barrier against moisture.

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