P-104 **Encapsulation of monacolin-k by spray drying**

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INTRODUCTION

Monacolin-k belongs to a family of metabolites from *Monascus rubber*, named as statins, and has raised the attention of health researchers due to its hypocholesterolemic activity. Monacolin K shows inhibitory activity over the enzyme 3-hidroxi-glutaril Co-A redutase, involved on the biosynthetic pathways of cholesterol (Hu 1997). Hypercholesterolemia is a major problem in public health in the world, being a main cause of death in several countries.

Monascus rubber has been employed for the production of food pigments, fermented foods, drinks and Chinese herbal medicine for centuries (Yang 2004).
The aim this work was to study the encapsulation of monacolin-k using the spray drying technique. The experiments were carried out following a Box-Behnken factorial design. Data was analyzed by the response surface methodology.

MATERIAL AND METHODS

Fermentation The strain of Monascus ruber van Tiegham (alt. Basipetospora rubra Cole & Kendrick), Tax (79, 373), supplied by Fundação André Tosello de Pesquisa e Tecnologia (Campinas - SP, Brazil), was preserved by spore growth in PDA medium with immobilization in silica beads and further drying under vacuum. The silica-immobilized strain was stored at 4ºC. The fermentation was run for 21 days. 2 mL of the monascus suspension was inoculated in 50 mL of a liquid medium (rice flour 30g/L, peptone 9g/L, glycerin 30g/L, glucose 110g/L, $MgSO_4$ 1g/L, KNO_3 2g/L, Agar 18g/L). The fermentation broth was filtered under low pressure, and the mycelium was separated, grinded and added to 100mL pure ethanol. This suspension was magnetically stirred for 48 hours and filtered to obtain the final extract.

Encapsulation study The encapsulation study followed a Factorial design Box-Behnken, with three factors and three levels. The influence of the following factors were studied: spray drying temperature, T (oC); drying adjuvant to monacolin k ratio, A/D (g/g) and incorporation time, t (min). The complete factorial design, the studied factors and their levels are shown in Table 1. Spray drying of monacolin solution was conducted using a laboratory-scale spray dryer model MSD 0.5 (Labmaq Ltda, Brazil). This is a bench scale apparatus with drying chamber and cyclone made of borosilicate glass, pneumatic (two fluid) spray nozzle and digital control of drying air flow rate, inlet and outlet temperatures. The following spray operational conditions were kept constant during all experiments: extract feed rate 5mL/min and drying air flow rate 1.25m³/min; atomizing air flow rate 50 L/min and pressure 2 Kg/cm². Maltodextrin (Corn Products Brasil Ltda, São Paulo, Brazil) with dextrose equivalent (DE) between 19 and 20 was used as encapsulating material. The proportion of maltodextrin and monacolin, A/D, was one of the factors studied.

Table 1 – Studied factors and their levels.

EXP	X_1	X_2	X_3	T (°C)	A/D (g/g)	t (min)
1	-1	-1	0	50	5	5
2	+1	-1	0	110	5	5
3	-1	+1	0	50	10	5
4	+1	+1	0	110	10	5
5	-1	0	-1	50	7.5	1
6	+1	0	-1	110	7.5	1
7	-1	0	+1	50	7.5	9
8	+1	0	+1	110	7.5	9
9	0	-1	-1	80	5	1
10	0	+1	-1	80	10	1
11	0	-1	+1	80	5	9
12	0	+1	+1	80	10	9
13	0	0	0	80	7.5	5
14	0	0	0	80	7.5	5
15	0	0	0	80	7.5	5

Chromatographic analysis of monacolin-k Extracts were analyzed by high performance liquid chromatography (HPLC Shimadzu, model SPD-10A), equipped with a Shimadzu pump model LC-10AT, diode array detector UV-Vis-DAD Shimadzu (model SPD-10A), and a chromatopac (model C-R6A). The column used was a reverse phase C-18, operating in isocratic mode with 1.0 mL/min of solution of acetonitrile/water (65:35). Monacolin k was detected at wavelength of 240 nm.

RESULTS AND DISCUSSION

The yield of microencapsulated monacolin-k is an important indication of process feasibility and of the adequateness of encapsulating material used for that specific drug. The values of microcapsules yield, Y%, varied from 7.0 to 69.0%. The highest yields observed, around 69.0%, may be considered an excellent result for spray drying at the bench top scale. The spray drying literature indicates yields that are rarely above 50%, due to the restrict size of drying chamber and the difficulty to establish the right amount of drying aids. The analysis of variance,

ANOVA, on the yield data was performed using the response surface methodology and the software Statistica 7 (Statsoft Inc, USA) and shows that the effects of the temperature and adjuvant/drug ratio, as well as their squared terms, were significant at levels varying from 0.5 to 5.0%. However, the incorporation time t, which is the mixing duration of monacolin-k and maltodextrin in solution, did not affect the yield. The effect of T and A/D on the yield can be seen in Figure 1, and prove the non-linear effects of these two factors on the yield. As shown in the response surface, the best yields were obtained for higher A/D and intermediate temperatures.

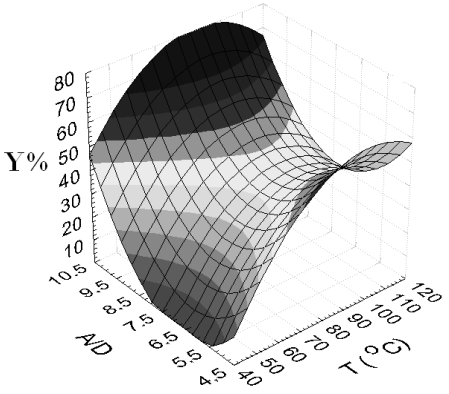

Figure 1 – Response surface of yield as a function of temperature and maltodextrin/monacolin ratio, A/D.

The chemical structure of monacolin K is identical to the one of lovastatin, as shown in Figure 2, a patented active drug against blood lipid dysfunction. The recovery of monacolin-k from microcapsules after the spray drying procedure was calculated with basis on the theoretical monacolin concentration expected in the powder. The monacolin-k concentration in the solution was 0.058% (w/w) in dry basis, and maltodextrin was added in the ratios shown in Table 1. The recovery of monacolin-k, R%, ranged from 21.6 to 40.1% for the 15 experiments of the Box-Behnken design. The results show that the monacolin-k losses were very important in most of the spray drying conditions. The thermal sensitivity of monacolin-k in aqueous solution was already reported, being very high for temperatures around 120°C (Ou 2009). However, in our experiments the temperature was not a significant factor to influence monacolin recovery, as demonstrated by the analysis of variance by surface response methodology.

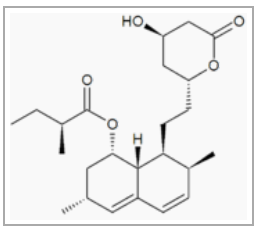

Figure 2. Chemical structure of monacolin-k.

The ANOVA proved that the quadratic term of the factor A/D affected monacolin recovery at the significance level of 5% ($p<0.0390$).

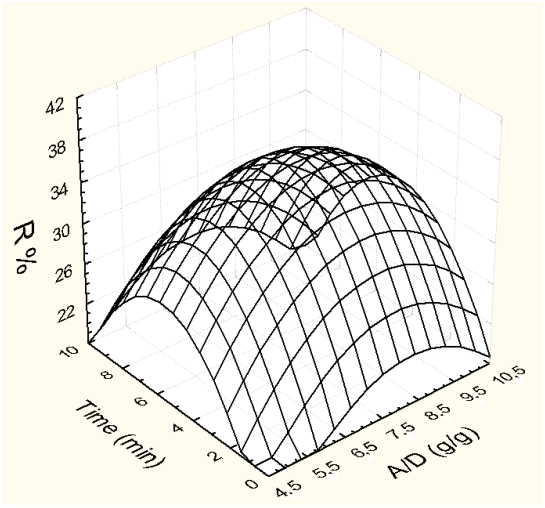

Figure 3. Response surface of monacolin-k recovery after microcapsules preparation by spray drying.

As can be seen in Figure 3, recovery has a maximum value at intermediary A/D ratios. Also, the same quadratic effect could be observed for incorporation time, t, but no with a significance level of 1% ($p<0.0072$). The highest recoveries were also found for intermediary values of t. The results indicate the complex behaviour of bioproducts during microencapsulation by spray drying (Jyothi 2010).

CONCLUSIONS

Despite the existence of many studies on the fermentation and biological activity of monacolin-k from *Monascus rubber* fermentation, none of them aimed at optimizing the microencapsulation process. In this work several parameters of the spray drying microencapsulation process were evaluated through a Box-Behnken factorial design. The microcapsules yield varied from 7.0 to 69.0%. The factors that affected drug recovery were the encapsulating material to drug ratio and the incorporation time. Unexpectedly, analysis of variance demonstrated that temperature did not affect the monacolin-k recovery. Based on these findings, the preparation of monacolin microcapsules by spray drying showed to be a feasible process, but strongly dependent on some spray drying parameters, which should be further investigated.

REFERENCES

- Hu, J.Q. (1997). *Production and application of red fermentation rice (Chinese)*. Light Industry Press, Beijing, China, 1 – 40.
- Yang, J.H. et al. (2004). *Storage stability of monascal adlay*. Food Chemistry, v. 90 p.303–309, 2004.
- Ou, H.P. et al. (2009). *Thermal degradation kinetics analysis of monacolin K in Monascus fermented products*. LWT Food Sci. & Technol, v 42(1) p. 292-296.
- Jyothi, N.V.N. et al. (2010). *Microencapsulation techniques, factors influencing encapsulation efficiency*. J. Microencapsulation, v. 27(3) p. 187-197.