

SPRAY DRYING OF LACTIC ACID CULTURES

II. The effect of culture conditions and storage on microorganisms survival

By

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SUMMARY

Effect of culture pH, incubation period, culture cold storage and additives on the survival of *S. lactis*, *S. thermophilus* and *L. bulgaricus* during spray drying were studied. Culture neutralization to pH 6.8 greatly helped the survival rate. Incubation periods longer than 24 hr as well as culture cold storage before drying detrimental to the microorganisms during drying. Out of the additives tried dextrin was superior. The survival rates obtained were 30%, 76% and 31% for *S. lactis*, *S. thermophilus* and *L. bulgaricus*, respectively.

The effect of the above factors as well as storage temperature on the survival of the dried microorganisms during storage were also studied. Storage temperature greatly affected the survival and activity of the culture. Storage at freezing temperatures preserved about 75% of the activity of the dried cultures.

INTRODUCTION

The interest in finding a large scale and inexpensive method for culture preservation led to the investigation of using spray drying (Sapp and Hedrich 1960, Foster 1962, Labuza et al. 1972 and Daemen and Van der Stege 1982). In this respect, Metwally et al. (1989) have investigated the effect of different parameters of spray drying on the survival rate of microorganisms who reported survival rates of 22%, 47% and 20% for *S. lactis*, *S. thermophilus* and *L. bulgaricus*, respectively.

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These results have encouraged us to pursue better survival rates through the investigation of other factors which affect these rates. This investigation was carried out to study the effect of culture conditions namely ; the pH, incubation period, cold storage and additives on the survival of *S. lactis*, *S. thermophilus* and *L. bulgaricus* during spray drying.

Also, factors that affect the survival of dried cultures during storage were studied. These are : the culture conditions before drying and the effect of storage temperature.

MATERIALS and METHODS

Cultures, equipments, methods of analysis and experimental procedures were as described by Metwally et al., (1989). Dextrin and sucrose additives were stirred into the culture at a rate of 2% (wt/vol) just before drying.

Dried cultures were sealed in polyethylene bags under atmospheric air and stored at 3 different temperatures.

RESULTS and DISCUSSION

A. Effect of culture conditions on microorganisms survival during spray drying :

1. The effect of culture pH :

At the end of the incubation period (24 hr) the culture pH was usually around 4.5. Table (1) shows the effect of culture pH on microorganism survival during drying. Neutralizing the pH to 6.8 greatly helped the survival during spray drying. As expected the effect of heat on microorganisms would be intensified at acidic pH's. Foster (1962) in his preliminary experiments on spray drying of *S. lactis* pointed out that neutralization re-

duced the activity. However, our results didn't agree with Foster's and we recommend culture neutralization. Actually, Foster in his preliminary experiments reported a survival rate of about 50% for *S. lactis* which was higher than whatever has been reported for spray drying.

2. Effect of culture incubation period :

Culture age and the phase at which the culture exists is before drying would affect the tolerance for heat (Foster, 1962).

The cultures were regularly incubated for 24 hr. and then spray

dried. In this experiment incubation periods of 24 and 40 hr. were used and the results are in Table (1).

Long incubation periods resulted in more destruction during spray drying. At the 24 hr. period, microorganisms would be within the active phase of growth (the log phase). It seems that microorganisms were more tolerable to heat in the active phase than in the stationary phase. Other workers (Foster, 1962 and Steel et al., 1983) agreed with these results when they freeze-dried microorganisms.

3. The effect of culture cold storage period :

The effect of culture cold storage period after incubation before drying was studied and the results are reported in Table (1).

Long periods of cold storage led to more destruction of microorganisms during spray drying. It is expected that long storage periods would lead the microorganisms into deep stationary phase. It seems that the microorganisms in this phase were more sensitive to heat during drying. It would be preferable then to dry the culture as it comes out of the incubator without delay.

4. The effect of additives :

Of the techniques used to spray dry heat labile substances is the so called encapsulation. This technique

depends on the addition of certain substances that would encapsulate the heat material to protect it against high temperatures. Two additives were investigated ; sucrose and dextrin and were added to the culture at a rate of 10% of the finished product (Table 1).

Sucrose did not improve the survival rate, so it was not fully investigated. On the other hand, Dextrin greatly helped microorganisms survival. The survival rates reached 30%, 76% and 31% for *S. lactis*, *S. thermophilus* and *L. bulgaricus*, respectively. These figures were much higher than what have been reported except for the preliminary results of Foster (1962) who claimed a 50% survival for *S. lactis* using a bench spray drier.

B. Factors affect spray dried microorganisms during storage:

1. Effect of storage temperature :

Table (2) illustrates the effect of storage temperature. Frozen temperature preserved the activity of the microorganisms best. Refrigerated temperature was somewhat reasonable. Room temperature was detrimental.

2. Effect of culture conditions before drying :

Table (3) shows the effect of culture incubation period and additives before drying on the survival

Table (1) : The effect of culture conditions on the survival of lactic acid bacteria during spray drying.

Culture conditions	microorganisms survival, %		
	<i>S. lactis</i>	<i>S. thermophilus</i>	<i>L. bulgaricus</i>
pH :			
4.6	2.2	11.80	1.14
6.8	6.2	18.00	5.42
Incubation period :			
24 hr	21.90	16.80	19.5
40 hr	6.60	11.50	9.1
Cold storage after incubation :			
24 hr	21.9	16.80	19.50
4 days	16.1	7.60	15.20
Additives :			
without	22	46	19
with Dextrin	30	76	31

- pH of all cultures were 6.8, unless other wise stated.
- Spray drier parameters : — inlet air temperature 190-200°C.
- outlet air temperature 70°C.
- two-fluid atomizer with 2.5 Kg/cm² air pressure.
- Two percent Dextrin was added to the culture before drying.

Table (2) : Effect of storage temperature on the survival of spray dried lactic acid bacteria

Microorganism	storage temperature		
	Room (20-25°C)	Refrigerated (5-7°C)	Freezing (-15°C)
<i>S. lactis</i>	3.60	50.00	57.10
<i>S. thermophilus</i>	7.00	50.00	75.00
<i>L. bulgaricus</i>	1.18	16.30	54.50

- Storage period : 30 days.
- Culture pH was 6.8 before drying.

Table (3) : Effect of culture condition before drying on the survival of dried lactic acid bacteria during storage.

Culture Conditions before drying	microorganisms survival after storage, %		
	<i>S. lactis</i>	<i>S. thermophilus</i>	<i>L. bulgaricus</i>
Incubation periods :			
24 hr	91.5	61.0	26.6
40 hr	87.5	69.9	74.2
Cold storage after incubation :			
without	91.50	61.0	26.6
4 days	93.40	80.0	30.0
Additives :			
without	37.6	30.5	12.5
with Dextrin	42.6	35.3	44.2

— Storage was for 30 days in the freezer.

— Culture pH was 6.8 before drying.

— Storage temperature was -15°C .

of microorganisms after 30 days of storage. *S. lactis*, while *L. bulgaricus* was the least tolerable (Table 2).

Incubation period and cold storage of the culture showed no clear effect on the survival rates during storage. On the other hand dextrin improved the survival rate during storage.

The above experiments pointed out that storage temperature was the most important factor which determines microorganisms survival. This factor followed by the addition of dextrin. Type of microorganisms also had an effect, *S. thermophilus* tolerated the storage better than *S. lactis*, while *L. bulgaricus* was the least tolerable (Table 2).

In conclusion, the use of the drier parameters selected in the previous work (Metwally et al. 1989) combined with the culture conditions investigated in this paper resulted in a great improvement in the survival of microorganisms during drying and storage. We ended with 30%, 76% and 31% survival for *S. lactis*, *S. thermophilus* and *L. bulgaricus* respectively. The improvement was good enough for us to think that with more experiments we could reach survival rates very close to what has been reported for freeze drying.

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حفظ مزارع بكتريا حمض اللاكتيك بطريقة التجفيف بالريزاذ

٢ - دراسة تأثير ظروف المزرعة البكتيرية والتخزين على

معدل بقاء ونشاط بكتيريا حمض اللاكتيك

حيث أجريت عملية تجفيف بالريزاذ لثلاث مزارع من بكتيريا حمض

اللاكتيك هي : *S. lactis*, *S. thermophilus*, *L. bulgaricus*

وتم دراسة تأثير كل من :

أولاً : ظروف المزرعة البكتيرية قبل التجفيف من حيث :

pH - فترة تحضين المزرعة - مدة حفظ المزرعة - إضافة الدكسترين

ثانياً : ظروف المزرعة البكتيرية بعد التجفيف والتخزين حيث تم تخزين

المزارع المجففة لمدة ٣٠ يوماً ودراسة تأثير حرارة التخزين وظروف المزرعة

قبل التجفيف .

وذلك على معدل بقاء ونشاط بكتيريا حمض اللاكتيك في المزرعة المجففة

وكانت أهم النتائج المتحصل عليها ما يلي :

أولاً : من حيث ظروف المزرعة البكتيرية قبل التجفيف :

١ - كان لدرجة pH المزرعة تأثير واضح على معدل بقاء ونشاط الخلايا البكتيرية للمزرعة حيث أدى معادلة الـ pH إلى ٨ إلى زيادة معدل البقاء بدرجة ملحوظة .

٢ - أدى إطالة فترة تحضين المزرعة إلى زيادة معدل إبادة بكتيريا المزرعة أثناء التجفيف .

٣ - كذلك أدى حفظ المزرعة لمدة أطول إلى خفض معدل بقاء ونشاط بكتيريا المزرعة بعد التجفيف .

٤ - كان لإضافة الدكستريين أثر فعال في وقاية الخلايا البكتيرية للمزرعة أثناء عملية التجفيف حيث وصل معدل البقاء للخلايا البكتيرية في المزرعة المضاف إليها الدكستريين إلى ٣٠٪ ، ٧٦٪ ، ٣١٪ في كل من *L. bulgaricus*, *S. thermophilus*, *S. lactis* على التوالي .

ثانياً : ظروف المزرعة البكتيرية بعد التجفيف والتخزين :

١ - أوضحت النتائج ما لدرجة حرارة التخزين من أثر فعال وملحوظ على معدل بقاء ونشاط المزرعة المجففة حيث أعطت المزارع المجففة والمخزنة تحت في الثلاجة ثم المخزنة في درجة حرارة الغرفة .

٢ - أعطت المزارع البكتيرية المضاف إليها دكستريين معدل بقاء أعلا في المزرعة البكتيرية الجافة بعد التخزين .

٣ - كان لنوع المزرعة البكتيرية تأثير على معدل بقاء ونشاط خلايا المزرعة بعد فترة التخزين حيث أعطت مزرعة *S. thermophilus* معدل بقاء عالى يليها *S. lactis* ثم *L. bulgaricus* .

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