BACTERICIDAL EFFECT OF THE SPRAY-DRYING SYSTEM FOR ANIMAL PLASMA ON TWO DIFFERENT E. COLI ANIMAL STRAINS

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INTRODUCTION

The spray-drying system is based on injecting a liquid product at high pressure into a drying chamber exposed to a hot air current. This liquid product is injected as an aerosol with very small diameter droplets. When these droplets come into contact with the hot air circulating inside the chamber, it causes very quick drying without damaging the physicochemical and organoleptic properties (Meerdink, 1993).
Although the spray-drying system is widely used for various food products (dehydrated dairy products, dehydrated eggs, soy protein, milk whey proteins, blood proteins and also dehydrated fruit and vegetable pulp), the literature available on this spray-drying system and its effects on the mortality of the microbial flora that may be present in the liquid product before spraying is very limited
Dried animal plasma for use in nutrition (as ingredient on meat products) is usually obtained from porcine or bovine blood. Porcine blood is obtained from healthy pigs in abattoirs under veterinary supervision. The most widely used system for collecting pig blood in Europe and USA consists of collecting the blood directly from the animal into a container, with continuous addition of an anticoagulant to prevent the blood from clotting. From the time the blood is collected in the container to spray-drying, the entire process usually takes place in a closed circuit that prevents external contamination of the blood or blood fractions. The only time the blood may contact the outside atmosphere is when the animal is slaughtered. From a microbiological hazard viewpoint, the most important bacteria that may enter the blood are those belonging to the Enterobacteriaceae family and this may be due to contamination of the animals' skin at the time of slaughter (Swingler, 1982; Parés and Carretero, 1997).

OBJECTIVE

The purpose of this study was to assess 1) the effectiveness of spray-drying on inactivation of *E. coli K88* and *E. coli K99* strains inoculated at 0.2% (w/v)from a bacterial concentrate in sterile pig plasma (22 – 24% solids), and 2) the effect of two spray temperatures (outlet air temperatures of 90ºC and 70ºC) on the inactivation of these two *E. coli* strains.

MATERIAL AND METHODS

Sterilization of pig plasma by ionizing radiation

One 25 kg bag of spray-dried pig plasma (SDPP) produced by APC EUROPE, S.A. was sterilized by ionizing radiation (5 Mrad), using Cobalt-60 as the source of irradiation. The final product's sterility was analyzed using the procedure described below.

Bacteria used in the study

All the bacterial strains (Table 1) were supplied freeze-dried. Nutrient Medium I (NM I), recommended by the suppliers, was used for reconstitution in all cases.
For each experiment, a new vial of frozen cells was used and 0.3 ml of culture were inoculated into 100 ml of sterile NM I. The cells were left to grow at 37ºC and 150 rpm for 18 h. The cells were subsequently concentrated by centrifuging (1000 x g for 20 min at 4 ºC) using sterilized 40 ml tubes containing 20 ml of culture media. The remaining culture media was removed by resuspending the cell precipitate in 20 ml of sterile 0.01 M phosphate buffer (PB). After resuspension, it was centrifuged again in the same conditions as described above and the resulting cell precipitate was resuspended again in 9 ml of PB.
For each test, SDPP sterilized at 5 Mrad was dissolved in autoclaved distilled water to a final concentration of 22 - 24% in solids. Half of this sterilized plasma solution was spray-dried to assay contamination produced during the spray-drying process (N_c). The other half was inoculated at the rate of 0.2% bacterial precipitate / 100 ml SDPP (N_f). The initial bacteria count of liquid plasma at 22-24% solid before spray-dried was also determined (N_i)

Table 1: Bacteria identification.

Bacteria	Supplier	Source	Suggested growth medium	Suggested Growing Conditions
E. coli K 88	Dr. Juárez. Department of Microbiology. University of Barcelona. Spain	Porcine alimentary tract	NM I	Aerobiosis, 37 ºC
E. coli K 99	E. coli 0101:K99 Nº 6012.Collection of Animal Pathogenic Microorganism (CAPM). Brno. Czech Republic.	Bovine alimentary tract	NM I	Aerobiosis, 37 ºC

Spray-drying

The solution containing SDPP at a concentration of 22 - 24% solids, with or without inoculation of 0.2% of bacterial precipitate, was spray-dried using a laboratory spray-drier (Büchi 190 Mini Spray Dryer). Inlet temperature was 215 ± 5ºC, and the outlet temperature was 90 ± 1ºC or 70 ± 1ºC. Air flow through the column and the suspension flow to the nozzle were set at 45 $m^3 h^{-1}$ (at 20ºC) and 0.2 lh^{-1}, respectively. The air flow through the feeding nozzle was set at 0.7 m^3h^{-1} (at 20ºC). Estimated dwell time was 0.41 s.

Bacterial mortality

Residual cell survival of processed samples (liquid plasma at 22-24% solids before dehydration and spray-dried samples) were determined using the standard plate count method.
In both cases, the number of Colony Forming Units (CFU) was determined after incubating plates at 37ºC for 48 hours. Results were expressed as CFU/g solids according to the equation: CFU/g = (CFU/ml) / [(% Solid contents of samples)/100].
The log reduction of the bacterial population exposed to spray-drying was calculated using the formula: Log_{10} reduction = $(N_f-N_c) / N_i$

Solid contents

The samples' water contents was determinined by weight loss after drying during overnight (18 h) in the convection oven at 105ºC.

RESULTS

There was a clear bacteria inactivation in both *E. coli* strains for all spray-dried plasma samples dehydrated at 215ºC inlet air temperature and 90ºC or 70ºC outlet air temperature. (table 2). The *K99* strain was more resistant to spray-drying than *K88* for both conditions assayed (Table 2). Dry matter of samples dried at 90ºC or 70ºC were 94.88 ± 1.07%. and 89.77 ± 1.56% respectively.

Table 2: Effect of outlet air temperature on the inactivation of each Enterobacteriaceae species.

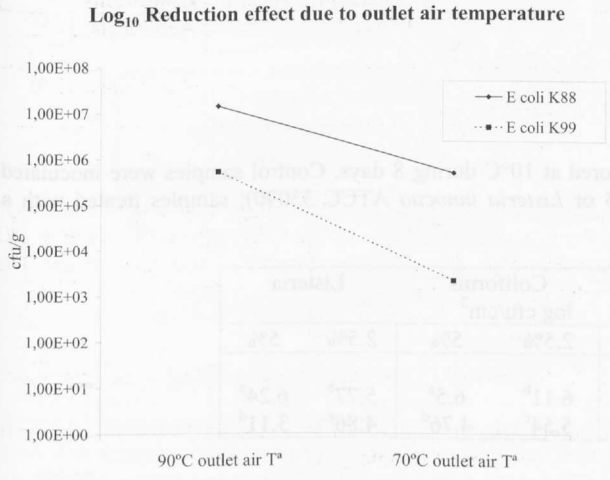
	E. coli K88	*E. coli K99*
90ºC Outlet air temperature		
Initial TPC (CFU/g) [a]	$7,4 \pm 0,5 \times 10^8$	$4,8 \pm 1,0 \times 10^8$
Final TPC (CFU/g) [b]	$5,3 \pm 0,7 \times 10^1$	$9,1 \pm 8,0 \times 10^2$
Log_{10} Reduction [c]	$1,4 \times 10^7$	$5,3 \times 10^5$
70ºC Outlet air temperature		
Initial TPC (CFU/g)	$4,1 \pm 1,4 \times 10^8$	$2,9 \pm 1,8 \times 10^8$
Final TPC (CFU/g)	$8,8 \pm 9,7 \times 10^2$	$1,4 \pm 1,7 \times 10^5$
Log_{10} Reduction	$4,7 \times 10^5$	$2,1 \times 10^3$

[a]= Mean ± SD of initial Total Plate Count (TPC) in inoculated plasma sample before spray-drying. (N_f-N_c) (n=4)
[b]= Mean ± SD of final Total Plate Count in inoculated plasma sample after spray-drying. (N_i) (n=4)
[c]= Average of Log Reduction calculated as (N_f-N_c) / N_i .

Differences in cell inactivation due to outlet air temperature

These data indicate that spray-drying outlet temperature is a key parameter in bacterial reduction and, therefore, in cell viability of the bacteria tested. Bacterial reduction increased at higher spray-drying outlet temperatures, with an estimated reduction between 1 and 2 log higher when operating at 90ºC (Fig. 1). These data are in agreement with Brian and Etzel (1997) who previously reported that outlet temperature is the most important parameter in cell survival during spray-drying.

Figure 1: Log_{10} reduction effect by spray-drying due to different outlet air temperatures (90ºC vs 70 ºC) in each *E coli* strain



DISCUSSION

Our laboratory results, obtained when operating in conditions similar to those occurring in industrial processes, indicate that when liquid plasma with a 22 - 24% solid contents and inoculated with 2 bacterial *E. coli* strains is exposed to a rapid drying process using the spray-drying system and an outlet air temperature of 90ºC, the cell viability of these two E. coli strains was significantly reduced, and bacteria count were decreased between 5 and 7 log. These results are consistent with tests performed in the animal plasma industrial processing plant, in which liquid plasma concentrated to a 22% solid contents and left for 24 h at room temperature to facilitate bacterial growth was found to have reduced Enterobacteriaceae contamination by 5.37×10^5 CFU/g after spray-drying with an inlet air temperature of 240ºC and an outlet air temperature of 90ºC (Polo, 2000, unpublished data).

According with Lievense et al. (1992) bacterial inactivation may be due to thermal inactivation and inactivation by dehydration, which take place simultaneously during the drying process. The ability of bacteria to survive drying depends both on its resistance to heat and dehydration and mechanisms to adapt to the rapid drying during the spray-drying process.

These results also indicate that the use of the spray-drying system when operating at high outlet air temperatures show a high bactericidal efficiency, similar to that of other mild bacterial inactivation systems such as pasteurization or UHT (Bridonneau et al., 1996; DSAT 2002).

CONCLUSIONS

The spray-drying system used to obtain edible blood products is highly efficient for inactivating *E. coli* strains (*E. coli K88 and E. coli K99*).

Bacterial inactivation obtained by the spray-drying system is dependent on the outlet air temperature, and increases progressively at higher temperatures.

REFERENCES

Brian, C.S. To, Etzel, M.R., 1997. Survival of *Brevibacterium linens* (ATCC 9174) after Spray Drying, Freeze Drying, or Freezing. J. Food Science 62 (1), 167-170.

Bridonneau, P. et al., 1996. Liquid Pasteurization of an Immunoglobulin Preparation without Stabilizer: Effects on its Biological and Biochemical Properties. Vox Sang 70, 203-209.

[DSAT] Dairy Science and Technology, 2002. Thermal Destruction of Microorganisms. University of Guelph. Available from: www.foodsci.uoguelph.ca/dairyedu/TDT.html. Accessed January 24.

Lievense, L.C. et al., 1992. Modelling the inactivation of *Lactobacillus plantarum* during drying process. Chem. Eng. Sci. 47, 87-97.

Meerdink, G., 1993. Drying of liquid food droplets. PhD thesis. Wageningen University.

Parés, D., and Carretero, C., 1997. La sangre de matadero: subproducto de la industria cárnica. Cárnica 2000. Agosto-Septiembre.

Swingler, G. R., 1982. Microbiology of meat industry by-products. In: Brown, M. H. (eds). Meat Microbiology. Applied Science Publishers LTD. London-New York. (pp. 179-224).