Effect of shear rate and oxygen stresses on the survival of *Lactococcus lactis* during the atomization and drying stages of spray drying: A laboratory and pilot scale study

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Abstract

The effect of shear rate and oxygen injury during atomization and the combination of these factors on the survival of *L. lactis* subsp. *cremoris* in spray drying was studied using laboratory and pilot scale spray dryers. The atomization was carried out using a two-fluid nozzle in the laboratory study and a two-fluid nozzle or rotary atomizer in the pilot scale study. The extent of oxygen-induced death was determined using ascorbic acid in the feed and atomizing the feed with gaseous nitrogen. The lowest levels of bacterial death were observed at lowest characteristic shear rate and in the presence of nitrogen and ascorbic acid. Quantitative analysis showed that lower shear rate, creating an oxygen-limiting environment during atomization and drying, and using oxygen scavengers in the feed were successful in enhancing bacterial survival in spray drying. We also report for the first time that, at least for *L. lactis*, the extent of death during the atomization stage far outweighs death during the drying stage, and that the majority of bacterial death (up to 93%) occurs during the atomization stage. The death of bacteria was found to be less when using a rotary atomizer or when using a two-fluid nozzle atomizer at lower flow rate. This work shows that bacterial death during spray drying can be minimized by using oxygen scavengers such as ascorbic acid and/or an anaerobic atomizing medium (such as nitrogen), and by altering the spraying conditions.

Keyword: atomization, spray drying, survival, shear rate, oxygen injury, *Lactococcus*

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1. Introduction

Drying is a widely used unit operation in the processing of dairy powders, pharmaceutical drugs, and other solutions and suspensions containing biologicals and bioactives such as proteins, enzymes, vitamins, antibiotics and micro-organisms. Spray drying is a well-established method to produce powders by rapid evaporation of solvents, especially water, and can be suitably designed and adapted to convert bacterial biomass, enzymes and other bioactive materials into powder form that can be stored for an extended period.

As indicated in the literature, the survival of bacteria during spray drying is lower than in some other preservation methods such as freeze drying (Chávez and Ledeboer, 2007; Santivarangkna et al., 2008). However, there are some notable advantages of spray drying over freeze drying including relatively low cost, ease of operation and ability to scale up to large throughput. These advantages associated with spray drying have been the major driving force in studies intending to optimize the spray drying process in order to achieve better bacterial survival. Altering the process conditions (especially using lower outlet air temperatures) and using suitable protectant matrices can greatly improve the survival of bacteria in spray drying (Chávez and Ledeboer, 2007).

The reasons for and implications of low survival of bacteria during spray drying and further low survival of spray dried bacteria during subsequent storage have been investigated to some extent (Silva et al., 2005; Teixeira et al., 1995). The inlet and outlet air temperatures used during the drying process and the composition of feed suspension are reportedly the major factors affecting the survival of bacterial biomass during spray drying and their subsequent biological activity in spray dried powders. Kitamura *et al.* (2009) showed that lower drying temperatures improved the microbial activity of spray dried probiotic food powders. Several factors can in principle contribute to bacterial death during spray drying including the extent of shear during atomization, dehydration during drying, thermal stress, and oxygen injury (due to the oxygen content of the drying medium and dissolved oxygen in the solution) (Riveros et al., 2009; Santivarangkna et al., 2008; Tymczyszyn et al., 2007). Atomization was not considered to be a significant cause of cell injury by these authors. The importance of removing oxygen from the drying medium has been emphasized only in limited instances (Sunny-Roberts and Knorr, 2009). These authors studied the applicability of

spray drying to produce *Lactobacillus rhamnosus* GG and *Lb. rhamnosus* E-97800 powders. They found that the survival of *Lb. rhamnosus* strains in the presence of monosodium glutamate (MSG) improved significantly which they attributed to the antioxidative properties of MSG. They suggested that MSG was instrumental in protecting the cell membranes during drying (Sunny-Roberts and Knorr, 2009). Using a laminar airflow system for single drop drying (Adhikari et al., 2007), Ghandi *et al.* (2012b) have reported that the presence of oxygen scavenger (sodium ascorbate) improved the survival of *Lactococcus lactis* throughout the drying history of a droplet, with estimates of 17-26% of bacterial death (depending on other conditions) being prevented by the addition of this antioxidant.

Atomization of the feed liquid has a direct and indirect effect on the biological activity. Extensional and shear stress is believed to play a direct role in the mortality of microbes. The atomization process also determines the initial droplet size distribution, which can affect the bacterial survival by altering the droplet and particle temperature and moisture trajectories, and altering oxygen exposure by changing the surface:volume ratio. Fine droplets typically experience more rapid drying and subsequently reach a higher temperature than coarser ones. The spray drying process is generally considered to be a single unit process when studying bacterial survival. To date, no studies have been reported that consider the survival or death of bacteria in the individual process stages of atomization (spraying) and drying of the resultant droplets. In addition, in most of the published literature, survival studies have been carried out in laboratory scale spray dryers; relevant investigations at pilot scale are rarely found. Comparative studies on the extent of death in pilot scale and laboratory scale laboratory spray dryers, especially due to shear and oxygen related stresses, are not reported in the literature. In order to fill the gap in knowledge of these two aspects we have quantified death/survival of the dairy fermentation starter bacterium *Lactococcus lactis* in the atomization and drying stages of spray drying in both laboratory and pilot scale spray dryers. The pilot scale spray drying trials were carried out to demonstrate whether the extent of bacterial death occurring in a laboratory scale laboratory spray dryer also occurred in a pilot scale spray dryer when the mechanism of atomization (twin fluid nozzle, atomization by air) was the same.

In this context, we had two key objectives in mind while undertaking this study. Firstly, to investigate the extent and the mechanisms of bacterial death in the spray drying process by quantifying the death of the bacteria in the atomization (spraying) process as well as in the drying process. Secondly, to quantify, compare and contrast the death of bacteria in

laboratory scale and pilot scale spray dryers. Furthermore, the adverse effect of oxygen during both the spraying and drying stages of the spray drying operation was investigated in this study.

2. Materials and methods

2.1. Materials

Lactococcus lactis subsp. *cremoris* strain ASCC930119 (Pillidge et al., 2009)from the Dairy Innovation Australia culture collection was cultured overnight in 15 ml of M17 broth (Oxoid, Australia) at 30°C under static conditions. The resulting culture was transferred to 1 L of M17 broth at 30°C under the same conditions. Cells were harvested by centrifugation at 2500×g for 5 minutes and then suspended in lactose:whey protein isolate (3:1) solution using gentle agitation at 25°C and adjusted to *ca.*10^{10} cells/ml.

α-Lactose monohydrate (99.8% purity, Sigma-Aldrich, Australia), whey protein isolate (WPI, protein content 94.5%, obtained by courtesy of Murray Goulburn Cooperative, Australia) and L-ascorbic acid (Chem-Supply, Australia) were used as received. Compressed Nitrogen (Gas code: 034, high purity) from BOC (Australia) was used in this study.

2.2. Methods

2.2.1.Solution preparation

Lactose:WPI (3:1; total solids 35% w/w) in deionized water was prepared by heating at 45±1°C and gently agitating with a magnetic stirrer. For experiments in the presence of ascorbic acid, a 10% (w/w) ascorbic acid solution was added to the bacterial suspension (in lactose:WPI, pH=6.5±0.1) just before spray drying, to a concentration of 0.7% (w/w) and pH 5.5.

2.2.2. Spray dryer operation

Spray drying was carried out in both laboratory scale and pilot scale spray dryers. Preliminary spray drying experiments were conducted to determine inlet and outlet air temperatures that gave acceptable bacterial survival and residual moisture content not exceeding 5% (w/w); inlet and outlet air temperatures of 130°C and 65°C, respectively, were chosen.

Laboratory spray drying was carried out using a Buchi 290 dryer (Buchi, Switzerland) with water evaporation capacity of 1 L/h. The bacterial suspension was pneumatically atomized using a two-fluid nozzle (nozzle diameter, inner D_i =1.2 mm; outer D_o=1.4 mm). In normal operation, the flow rate of the drying air was maintained at 35 m^3/h while the aspiration was at 100%. Characteristic shear rate was manipulated by altering the flow rate of atomizing air (or nitrogen) through the nozzle. In experiments to quantify the effect of shear without drying, the bacterial feed suspension was sprayed into a sterile bottle (*i.e.* not into the standard drying chamber).

The spray dryer used for the pilot scale study was a Drytec Spray Dryer (Drytec, England; water evaporation capacity: 8 L/h; exhaust fan capacity: 160 m^3/h) fitted with a rotary wheel atomizer or a two-fluid nozzle (inner D_i =1.6 mm; outer D_o=2.4 mm; atomizing air pressure: 550 kPa).

The dried powder was collected in a product container connected to the bottom of the cyclone separator, continuously cooled using an electric fan to minimize bacterial death.

2.2.3.Calculation of characteristic atomization shear rates

Hede *et al.* (2008) have written an excellent review of two-fluid atomization, focusing on the effect of formulation, nozzle geometry and feed and gas flow rates on the resultant droplet size distribution(Hede et al., 2008). Flow in the atomizer is extremely complex, and an estimation of the shear rate and stress is difficult to make. The multiphase computational fluid dynamic approach has been used to characterize flows in the vicinity of a two fluid nozzle (Beheshti et al., 2007; Deux and Sommerfeld, 2006). This approach can be used to quantify the shear stress and also to estimate the initial droplet size distribution. Fig. 1 shows a schematic diagram of an external mixing type two-fluid nozzle.

For simplicity, a characteristic shear rate in an atomization process can be estimated as follows, based on the analysis of Hede *et al.* (2008). Assuming momentum is transferred between the liquid and gas in the mixing zone and both leave the atomization zone at constant velocity, the average velocity (v_{av}) ($m.s^{-1}$) can be calculated using equation (1).

$$v_{av} = \frac{v_g \cdot \dot{m}_g + v_L \cdot \dot{m}_L}{\dot{m}_g + \dot{m}_L} \qquad (1)$$

v_g and v_L are the velocity of gas and liquid ($m.s^{-1}$), respectively. These two velocities are calculated at the point of atomization. The gas velocity is calculated based on density at prevailing absolute pressure and the cross-sectional area of the annulus through which the

atomizing gas flows. The liquid velocity is calculated using a measured density of 1105.92 ± 3.63kg/m^3. The $\dot{m}_g$ and $\dot{m}_L$ are the mass flow rates of gas and liquid (kg.s^{-1}), respectively. An estimate for the characteristic shear rate at a two-fluid nozzle atomizer can be made by equation (2) given below (Hede et al., 2008).

$$\dot{\gamma} = \frac{2(v_{av} - v_L)}{D_L} \quad (2)$$

where ($\dot{\gamma}$) is the characteristic shear rate (s^{-1}) and D_L is the inner nozzle diameter (m).

The characteristic shear rate in a rotary wheel atomizer can be calculated as suggested by Garcia *et al.* (1997), represented by equation (3) (Garcia et al., 1997).

$$\dot{\gamma} = 0.8\, R \sqrt{\frac{\rho\ (2\pi N)^3}{\mu}} \quad (3)$$

where, R is the radial distance from the centre to the edge of the disc (in this case: 0.02675 m); ρ and μ are the density (kg.m^{-3}) and viscosity (Pa.s) of the liquid, and N is the rotational velocity of the disc (20,000 rpm, expressed as 333.33 revolution.s^{-1}). The relevant parameters used while calculating the characteristic shear rate are presented in Table 1.

2.2.4. Enumeration of bacterial survival in spray dried powders

Survival of *L. lactis* was determined on fresh, sprayed and dried cultures by counting live and dead cells using a fluorescein diacetate (FDA) staining method (Lentini, 1993). The initial viable cell concentration was determined while the spray drying process was continuing, and the viability of dried cells was assessed after rehydrating 0.1 g of spray dried powder in 9.9 mL sterile peptone water (1% w/v, pH=7±0.1) (Gardiner et al., 2000; Ghandi et al., 2012a). The bacterial survival in cell suspensions and powders was calculated from live cell counts expressed cell per gram dry solid. Live cell counts in the fresh feed suspension was defined as 100%.

2.2.5. Moisture content and water activity

The moisture content of the powder was determined by drying the sample in a vacuum oven (Thermoline Scientific, Australia) at 65°C for 24 h (Adhikari et al., 2009). The samples were allowed to cool to room temperature in desiccators containing silica gel. The water activity of the powder was determined using a water activity meter (Novasina,

Switzerland) at ambient temperature (22.5±0.5°C).

2.2.6.Statistical analysis

Statistical analysis was performed using ANOVA. The mean values and standard deviation were calculated from triplicate experimental data. Differences were compared by Tukey test for all experiments and considered significant at $p<0.05$. All statistical analyses were performed with MINITAB (Minitab Statistical Software, Release 15).

3. Results and discussion

The results have been organized in two main sections. Section 3.1 addresses the effects of shear, nitrogen atomization and presence of oxygen scavenger on bacterial survival in laboratory spray drying experiments, and pilot scale studies are presented in Section 3.2.

3.1. Bacterial death during atomization and drying stages of laboratory scale spray drying

3.1.1. Effect of shear rate and oxygen level

The prevailing high shear rates in the atomization process have potential to cause cellular injury and bacterial death. However, published studies are either silent on this aspect or suggest that the death due to shear during atomization is negligible (Johnson and Etzel, 1993; Santivarangkna et al., 2008). Bacterial death during spray drying is widely considered to occur solely during the drying stage, mainly due to thermal and dehydration induced stresses but with some evidence of the involvement of oxygen-induced damage (Ghandi et al., 2012a, b).

We selected three progressively increasing characteristic shear rates (166×10^3 s^{-1} at 116 kPa, 229×10^3 s^{-1} at 124.3 kPa and 559×10^3 s^{-1} at 142.3 kPa atomizing gas upstream absolute pressure) and two atomizer flows (air or nitrogen) to examine the extent of bacterial death in the atomization stage of a laboratory dryer (*i.e.* spraying without drying). Fig. 2 presents the survival of bacteria at these three shear rates, showing that survival is greatly decreased during atomization at increased characteristic shear rate, whether the atomization was carried out using compressed air or gaseous nitrogen. Higher bacterial survival was observed at the same shear rates when nitrogen was used as the atomizing medium.

These observations confirm shear-related cell damage during spraying, presumably through cell disruption (Goldberg, 2008)and at the molecular scale by causing irreversible protein denaturation(Bekard et al., 2010; Goldberg, 2008) , and further implicate oxygen as an

additional contributor to bacterial death. Shear and oxygen damage are likely to be inter-related. As shear is increased, the resulting degree of oxygen damage would be expected to be influenced by the decreasing average droplet size and its effect on the surface:volume ratio. It might also be that shear-damaged cells are more vulnerable to oxygen, but the current experiments do not provide sufficient detail to explore these points to quantitative conclusions.

We extended this approach and used nitrogen as the atomizing medium and also incorporated ascorbic acid in the feed as an oxygen scavenger (Fig. 3). As in Fig. 2, the extent of bacterial death increased when the characteristic shear rate was increased. This was true whether atomization was carried out using compressed air or nitrogen gas, but the extent of death was lower when nitrogen was used as the atomizing medium and when ascorbic acid was added. The lowest level of bacterial death (38.4%) was observed at the lower characteristic shear rate (229×10^3 s^{-1}), using nitrogen atomization in the presence of oxygen scavenger, and the highest (60.6%) at the higher characteristic shear rate (559×10^3 s^{-1}) with air atomization and without oxygen scavenger. These observations further confirm that controlling the shear rate, creating a low-oxygen environment and using an oxygen scavenger in the feed enhances bacterial survival during atomization. Combination of nitrogen and ascorbic acid resulted in even greater survival than either alone. This combination eliminates oxygen from the atomizer gas-flow and protects against oxygen and its radicals (including oxygen already dissolved in the bacterial suspension prior to drying and in the headspace of the sample collection bottle). Ascorbic acid is also able to protect against a variety of non-oxygen oxidizing radicals (Buettner and Jurkiewicz, 1996) , but the importance of such species in cell death is not known.

3.1.2. Bacterial survival in spray drying

Fig. 4 presents the bacterial survival results of spray drying when air, nitrogen, and air or nitrogen plus ascorbic acid were used as atomizing medium. In all cases the feed contained 35% (w/w) lactose:WPI (3:1) and other spray drying conditions were identical. Not surprisingly, survival after spray drying (i.e. spraying and drying) was lower than after spraying (atomization) alone, but enhancement of survival similar to that seen in spraying alone was seen when using nitrogen atomization, ascorbic acid addition, or both.

Fig. 5 shows the results of a spray dying experiment extending these observations by combining the variables of shear rate, air/nitrogen atomization and addition of ascorbic

acid. The results confirm the influence of these factors and that they can be manipulated to improve bacterial survival. The total death in spray drying depends on the atomizer shear rate, medium used for atomization and whether or not oxygen scavenger is used in the feed. As can be seen from Fig. 5, a higher extent of bacterial death occurred in samples when compressed air was used for atomization compared to when nitrogen was used, regardless of the presence or absence of ascorbic acid. Similarly, a higher extent of bacterial death was observed in the absence of ascorbic acid compared to when ascorbic acid was incorporated in the feed solution, regardless of whether compressed air or nitrogen was used as an atomization medium.

As in spraying alone, the combination of both nitrogen and ascorbic acid was the most effective at both shear rates tested. For example, at the shear rate of 229×10^3 s^{-1} the death of the bacterial cells dropped from 61.9% to 45.9% (air drying) and 45.7% to 40.5% (nitrogen drying) after the addition of ascorbic acid. Similarly, improvement in bacterial survival was observed by Wang *et al.* (2006), improving the viability of *Bordetella pertussis* by 90% through the use of nitrogen as the drying medium in a spray-freeze-drying process(Wang et al., 2006).

Comparison of Fig. 3 and Fig. 5 shows that death of bacterial cells occurring in the drying stage is much lower than the death occurring in the atomization stage . This is an important finding, and is the opposite of the belief that the majority of bacterial death occurring in spray drying processes occurs during the drying stage, mainly due to thermal and dehydration stresses (Johnson and Etzel, 1993; Santivarangkna et al., 2008) and that death of bacteria due to high shear during the atomization process is negligible (Johnson and Etzel, 1995; Kim and Bhowmik, 1990). Our data show over 80% of bacterial death occurring in the spray drying process occurred at the atomization stage.

Our results indicate that the two main factors contributing to bacterial death during spray drying are shear-induced injury during atomization and oxygen-induced injury. The extent of death observed during the atomization stage is presumably a consequence of the interplay of oxygen and prevailing shear rates rather than these factors acting alone. In other words, the survival of bacteria during spraying depends on both the severity of atomization and the degree of oxygen injury, and these can be ameliorated to some extent by excluding air, adding an oxygen scavenger and altering the spraying (atomization) conditions.

3.2. Bacterial death occurring in pilot scale spray dryers

3.2.1. The influence of atomization on bacterial death at pilot scale

Two different atomizers (rotary wheel atomizer and two-fluid nozzle) having different atomizing mechanisms and quite different characteristic shear rates were used to investigate the effect of shear rate on death of bacterial cells during the atomization stage. The survival data of the bacteria after atomization (*i.e.* without drying air) and at the end of spray drying at pilot scale are presented in Fig. 6. Ascorbic acid was also used in some of the feed solutions to study the effect of oxygen scavengers in the atomization and drying stages.

Consistent with observations at laboratory scale, Fig. 6 and Table 2 show that survival of the bacteria was lower at higher shear rate (determined by the different nozzles used) and significantly higher in the presence of ascorbic acid in the feed ($p<0.05$). Hence, our findings that a substantial degree of bacterial death occurs during the atomization stage are not limited to the laboratory spray dryer.

3.2.2.Bacterial death in spray drying at pilot scale

As can be seen from Fig. 6 and Table 2, the effects of increasing shear rate (shear induced death) and the presence or absence of oxygen scavenger (oxygen-induced death) can be clearly observed in pilot scale spray drying. Survival after drying followed the same trends as survival after spraying and also supported the observations at laboratory scale. The highest survival was achieved when the rotary atomizer (lowest shear rate) was used together with ascorbic acid as oxygen scavenger. On the other hand, the highest level of death was observed when the two-fluid nozzle (highest shear rate) was used for atomization and no antioxidant was added to the feed.

Table 2 summarizes the pilot scale trials. Crucially, most of the observed bacterial death occurred at the atomization stage rather than the drying stage. Furthermore, the presence of ascorbic acid reduced bacterial death. It can further be estimated that the shear-induced death of bacteria (assuming shear to be the cause of all death occurring in the presence of ascorbic acid) ranged from 40.2% (rotary wheel atomizer) to 70.1% (two-fluid nozzle). On the other hand, death in the drying stage only ranged from 7.5% to 13.3%. The oxygen scavenger was found to enhance bacterial survival in both the atomization and drying stages of spray drying.

3.3. Comparison of bacterial death in laboratory and pilot scale spray dryers

As two-fluid nozzles were used for atomization in both laboratory and pilot scale trials, it allowed comparison of the atomization and drying stages in both laboratory and pilot scale operations.

Table 3 shows that higher bacterial death occurred at pilot scale at both the atomization and drying stages. This can be attributed to higher shear rates prevailing in the pilot scale spray dryer during the atomization process, causing physical damage and creating smaller spray drops that will be prone to higher oxygen levels and more rapid heating. In both dryers the majority of bacterial death occurred during atomization rather than during drying; bacterial death at the atomization stage was five to eleven times higher than that in the drying stage. Ascorbic acid was similarly effective in enhancing the bacterial survival at pilot scale as it was at laboratory scale. For example, with the two-fluid nozzles, ascorbic acid addition reduced bacterial death upon atomization decreased from 60.6% to 56.2% (laboratory scale) and 78.9% to 70.1% (pilot scale) and after drying from 13.9% to 5.1% and 12.3% to 9.1%, respectively.

Higher bacterial death was observed in pilot scale operation (79.2% to 91.2%, Fig. 6) compared to laboratory scale operation (45.9% to 74.5%, Fig. 5) when the same type of atomizer (albeit with different shear properties) and feed composition were used. The majority of bacterial death was found to be occurring in the atomization stage (average 89% of total death) rather than in the drying stage (11%). The type of nozzle used (or, more generally, the shear associated with the nozzle and how it is used) was found to significantly affect bacterial survival during the atomization process. For example, at pilot scale, 79.2% to 91.2% bacterial death occurred with a two-fluid nozzle while 45.8% to 71.1% bacterial death occurred with a rotary wheel atomizer.

3.4. The moisture content and water activities of spray dried powders

The moisture content and water activity values are of great importance in determining the shelf-life of particulates and powdered products and it is generally accepted that the higher the moisture content and water activity; the shorter will be the shelf-life. The moisture content and water activities (a_w) of the spray dried powders are summarized in Table 4. It can be seen from this table that the moisture content of the powders obtained from pilot scale spray drying trials showed overall lower values (3.91%-4.11%, w/w) than those powders obtained from the laboratory scale spray dryer (4.81%-5.19%, w/w). Similarly, the

water activity values were lower for powders obtained from the pilot scale spray dryer. When air was replaced by nitrogen as the atomizing medium, both the moisture content and water activities of the spray dried powders increased significantly ($p<0.05$), perhaps indicating higher relative humidity of the nitrogen gas used in the experiment. However, these moisture content and water activity values all fall within the range typical of industrially produced spray dried food powders (Bhandari and Adhikari, 2008).

4. Conclusions

The effects of characteristic shear rate and the extent of oxygen-induced injury in the death of a *Lactococcus lactis* bacterial culture in both laboratory and pilot scale spray drying have been investigated. Death was found to occur due to shear and oxygen-induced injuries, in addition to the thermal and dehydration-related stresses generally associated with drying. However, death occurring due to thermal and dehydration-related stresses was found to be much lower than death occurring due to shear and oxygen effects.

Bacterial death due to oxygen-injury could be minimized by using ascorbic acid as an oxygen scavenger, using nitrogen (instead of air) as the atomizing medium or, for best results, a combination of both. This study indicates a previously unappreciated opportunity to improve the efficacy of spray drying of micro-organisms through engineering and chemical approaches to lowering shear forces and operating under low-oxygen conditions.

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Table 1.

Parameters used to calculate the characteristic shear rate in laboratory and pilot scale dryers used in this study.

Parameter	Laboratory-scale		Pilot Scale	
	Liquid	*Gas*	*Liquid*	*Gas*
$\dot{m}$ (kg/s)	[a] 8.33×10^{-5} [b] 1.67×10^{-4} [c] 2.01×10^{-4}	[a] 1.08×10^{-4} [b] 1.80×10^{-4} [c] 3.13×10^{-4}	8.17×10^{-4}	2.92×10^{-1}
ρ (kg/m^3)	1105.92± 3.63	1.2039*	1105.92 ± 3.63	1.2039*
μ (Pa.s)	0.0564± 0.006		0.0564 ± 0.006	
D_i (m)	0.0012		0.0014	
D_o (m)	0.0014		0.0024	
Volumetric flow rate (L/h)	[a] 0.3 [b] 0.6 [c] 0.9	[a] 283 [b] 439 [c] 667	2.66	1.6×10^{5}
Pressure drop (ΔP_d) (Pa)		[a] 1.5×10^{4} [b] 2.3×10^{4} [c] 4.1×10^{4}		4.487×10^{5}

[a,b,c], refer to three different processing spray drying conditions with different characteristic shear rate.

* assumes ideal gas law, where $P=101325+\Delta P_d$ (Pa).

Table 2.

Death of bacteria at various stages of pilot scale spray drying.

	Rotary wheel atomizer (characteristic shear rate = 182×10^3 s^{-1})		Two-Fluid nozzle (characteristic shear rate = 145×10^6 s^{-1})	
	Without Ascorbic acid	With Ascorbic acid	Without Ascorbic acid	With Ascorbic acid
Death at atomization stage (%)	63.59 ±1.25	40.18 ±1.15	77.89 ±1.55	70.11 ±1.28
Death at drying stage (%)	7.5 ±0.64	5.6 ±0.44	13.28 ±0.86	9.1 ±0.43
Total death in the spray drying process (%)	71.09 ±1.34	45.78 ±1.32	91.17 ±1.64	79.21 ±1.54

Table 3.

Bacterial death at different stages of laboratory and pilot scale drying in air using a two fluid nozzle.

	Characteristic Shear rate (1/s)	Oxygen scavenger	Death at Atomization (%)	Total death at spray drying process (%)	Death at drying stage (%)
Two fluid (Laboratory scale)	559×10^3	-AA	60.62±1.18	74.51±1.21	13.89±0.65
		+AA	56.21±0.82	61.31±1.05	5.10±0.72
Two fluid (Pilot scale)	145×10^6	-AA	78.89±1.09	91.17±1.10	12.28±0.58
		+AA	70.11± 1.11	79.21±1.08	9.10±0.84

Table 4.

Moisture content and water activities of powders obtained from laboratory and pilot scale drying with two-fliud nozzles at different shear rates (AA, ascorbic acid).

Shear rate (Laboratory scale)	AIR		NITROGEN	
	Moisture content	*Water activity*	*Moisture content*	*Water activity*
229×10^3	4.81 ± 0.083	0.264 ± 0.005	6.12 ± 0.07	0.321± 0.005
229×10^3+AA	5.15 ± 0.032	0.286 ± 0.005	7.38 ± 0.06	0.519± 0.001
559×10^3	4.89 ± 0.027	0.252 ± 0.001	6.61 ± 0.12	0.481± 0.005
559×10^3+AA	5.19 ± 0.092	0.281 ± 0.005	6.58 ± 0.02	0.362± 0.003
Shear rate (Pilot scale)	Two-fluid nozzle			
	Moisture content	*Water activity*		
145×10^6-AA	3.91±0.192	0.22±0.003		
145×10^6+AA	4.11±0.074	0.24±0.002		

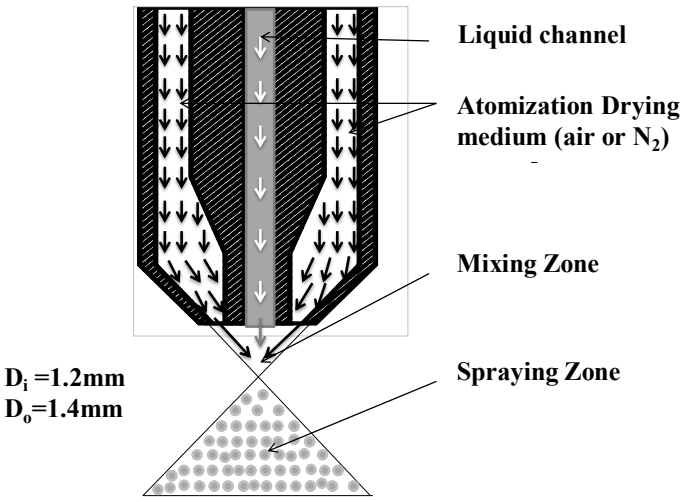

Fig. 1. Schematic diagram of external two-fluid nozzle.

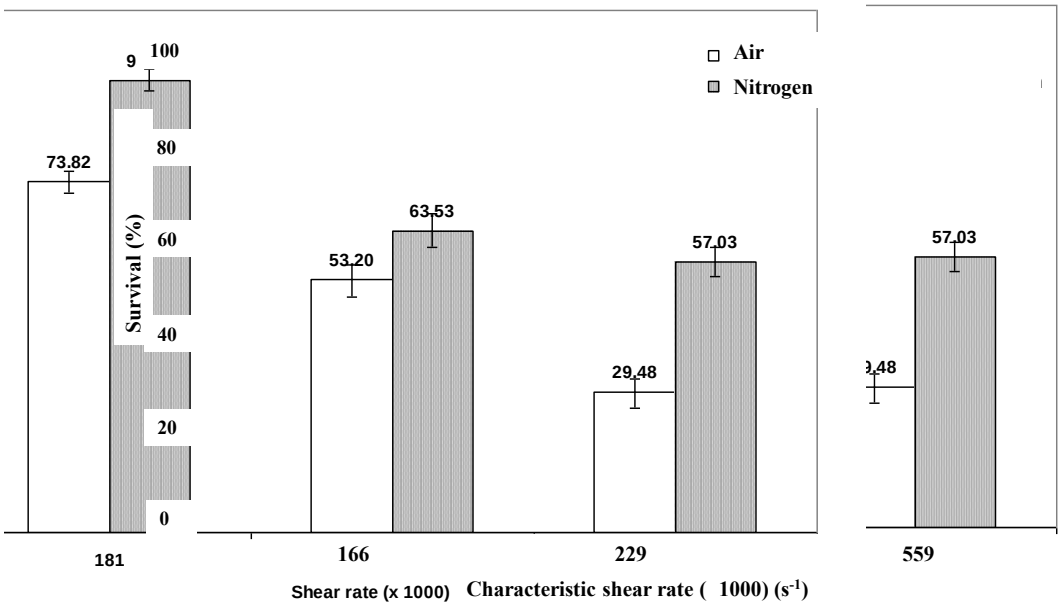

Fig. 2. Survival of *L. lactis* during atomization at shear rates of 166×10^3 s^{-1}, 229×10^3 s^{-1} and 559×10^3 s^{-1}. Atomization was carried out using compressed air or gaseous nitrogen.

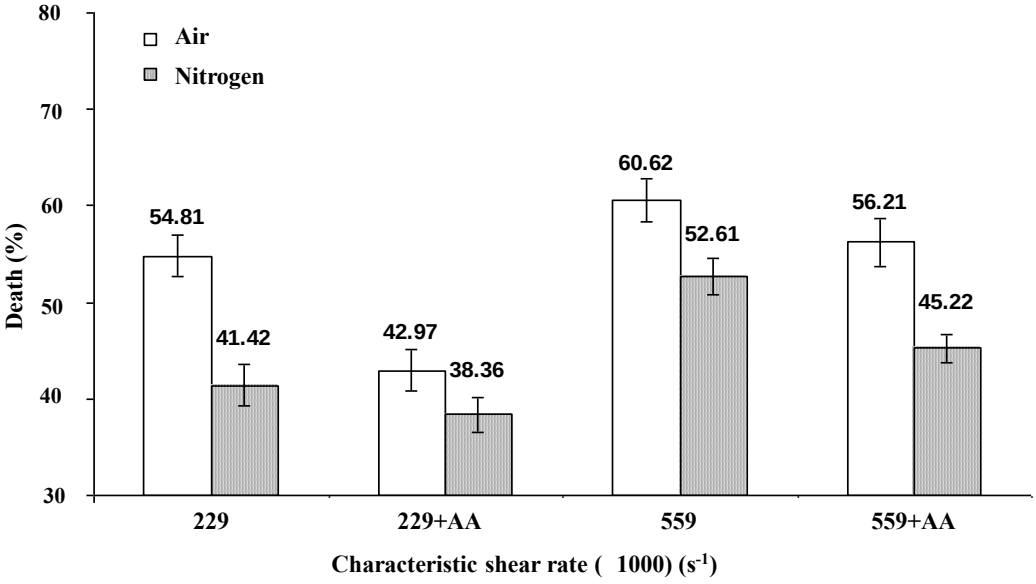

Fig. 3. Death of *L. lactis* in the atomization stage of spray drying with air or nitrogen, with or without antioxidant. AA; ascorbic acid.

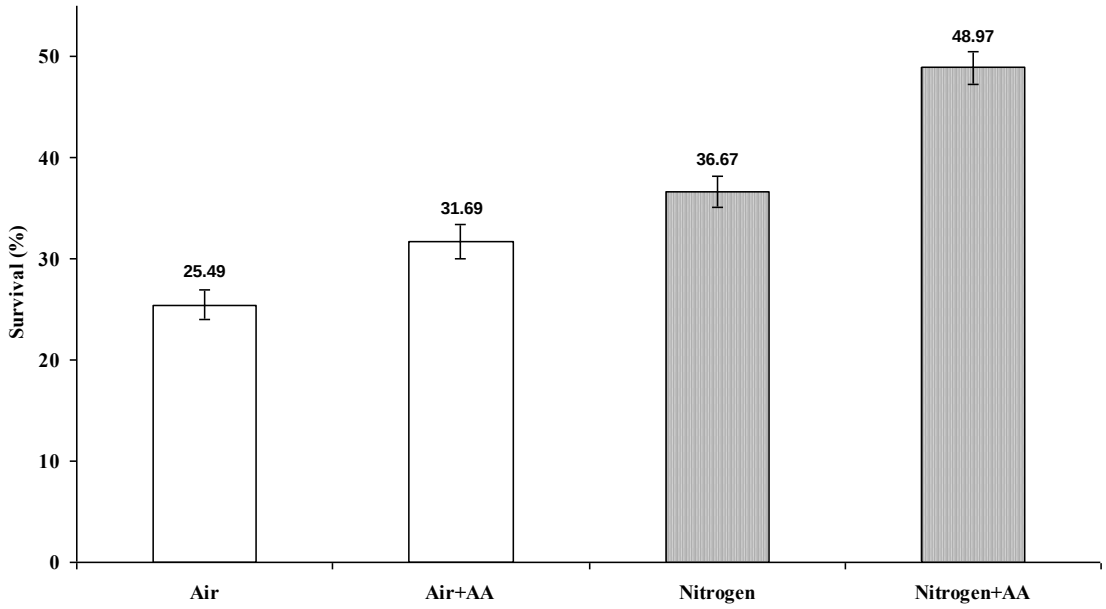

Fig. 4. Survival of *L. lactis* in spray drying with air or nitrogen as atomizing medium. AA; ascorbic acid. Spray drying temperatures: inlet, 130^{o}C; outlet, 65^{o}C.

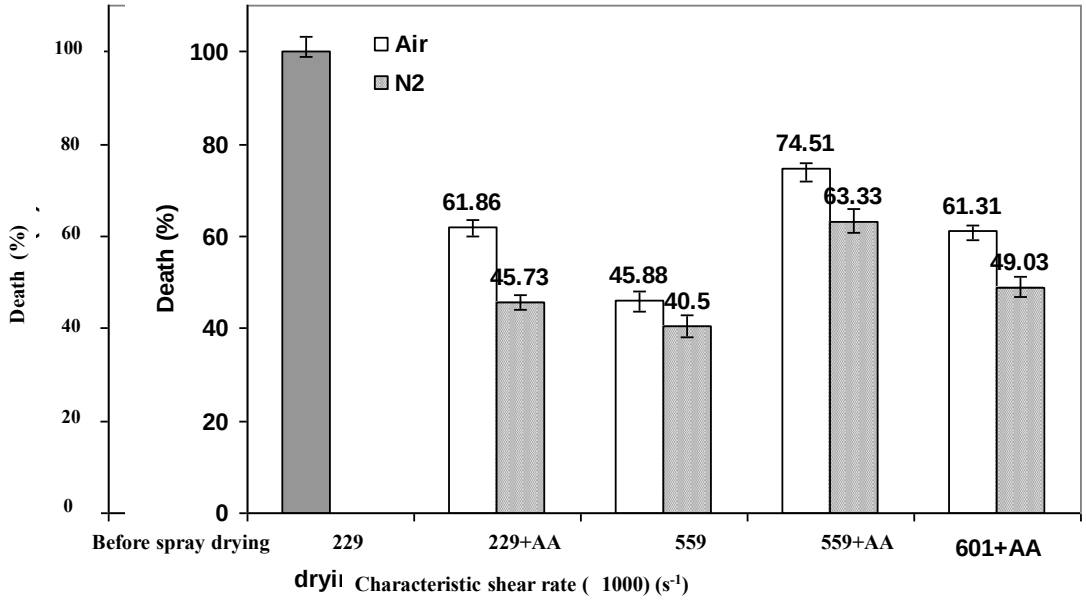

Fig. 5. Total death of *L. lactis* measured at the completion of spray drying, with conditions as for Fig. 4.

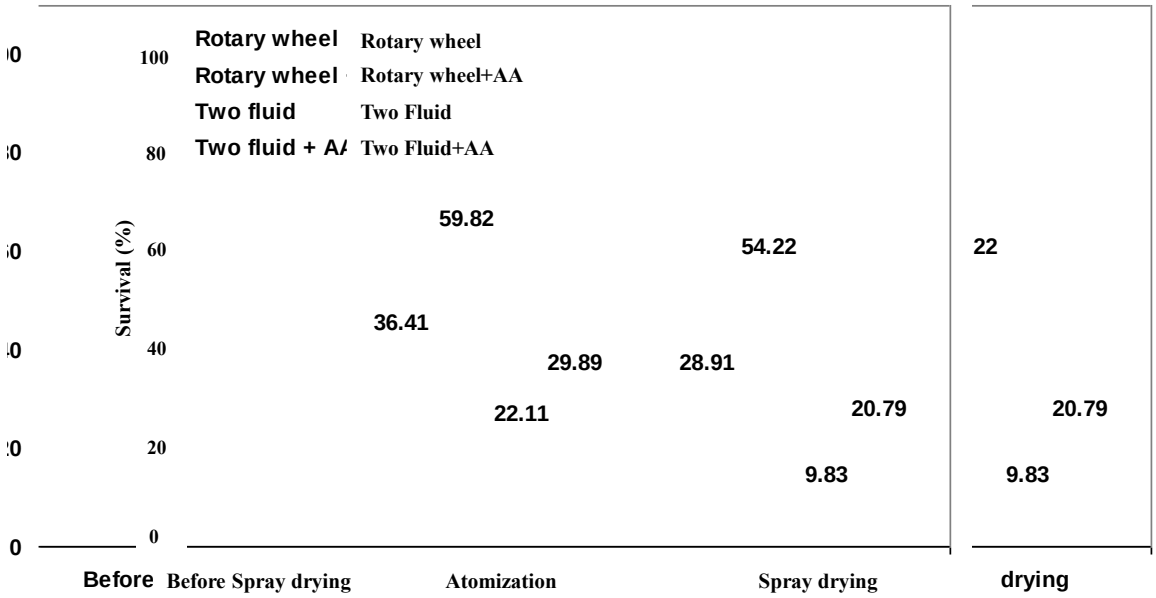

Fig. 6. Comparison of survival of *L. lactis* at pilot scale under different conditions (rotary wheel atomizer, two-fluid nozzle and presence or absence of ascorbic acid (AA).