Drying kinetics and survival studies of dairy fermentation bacteria in convective air drying environment using single droplet drying

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ABSTRACT

The drying and survival kinetics of *Lactococcus lactis subsp. cremoris* in convective air drying environment were measured using single droplet drying experiments. Tests were carried out at five different drying temperatures (45–95°C) at a constant air velocity (0.5m/s) and within 2.6–12% relative humidity. The effect of protective agents (10% (w/w) of lactose, sodium caseinate and lactose:sodium caseinate in the solid ratio of (3:1) was also evaluated. The thermal inactivation kinetics parameters in convective air drying and isothermal water bath heating were determined and compared. The results showed that the final temperature attained by the droplet affected the survival of the bacteria significantly. Lactose and sodium caseinate, as protective agents, enhanced the survival of bacterial cells significantly at all the test conditions. The lactose:sodium caseinate (3:1) mixture synergistically enhanced the survival of the bacterial cultures. At higher droplet temperatures (≥65°C) the bacterial cultures were inactivated by both the dehydration and thermal stresses. At lower droplet temperatures (≤ 55°C) the rate of change in droplet temperature had much stronger effect on the bacterial survival. The death of these bacteria followed the first-order kinetics during convective single droplet drying as well as during isothermal water-bath heating environments. However the inactivation energy in convective single droplet drying environment was found to be 10.8 times higher compared to that in the isothermal water bath heating environment within the medium temperature of 45-95^{o}C. This indicated that these bacteria can survive much better in the former than the latter environment.

Keywords: Single droplet drying, drying kinetics, survival kinetics, inactivation energy, *Lactococcus*, protectants

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1. Introduction

Most industrial dairy fermentation bacteria, especially the lactic acid starter cultures used in cheese and yoghurt production, are supplied in frozen or freeze dried concentrated forms. Although survival of frozen culture concentrates is excellent, frozen starter cultures have the disadvantage of requiring very low transportation and storage temperatures (preferably ≤ –40°C). Under some usage conditions, the thawing of the frozen cultures may require extra heat energy input and heightened sanitation, both of which eventually add up the production cost (Chavez and Ledeboer, 2007; Santivarangkna et al., 2007). Unless pelletized, frozen starter cultures do not readily offer the convenience of mixing two or more cultures in desired ratios while frozen.

Converting the starter cultures into dry particulate form has many advantages. The volume is considerably reduced and there is no need to use refrigerated transportation and storage and its handling becomes much easier. Freeze drying is a preferred drying method for thermally sensitive bacteria as it keeps their survival at a reasonably high level. For example To and Etzel (1997) found that the survival of three different species of bacteria (*Lactococcus lactis* subsp. *cremoris* D11, *Lactobacillus casei* subsp. *pseudoplantarum* UL137 and *Streptococcus thermophilus* CH3TH) varied between 60-70% and 0.35-34% during freeze drying and spray drying, respectively. However, freeze drying is a batch process with a considerably long drying time. It is also expensive due to high energy requirements (Santivarangkna et al., 2007). For drying of starter cultures, spray drying can be a viable alternative if the survival can be raised to make it economically attractive. This is because spray drying is relatively inexpensive, energy efficient, high throughput and a hygienic process. Furthermore, the operational and capital costs of spray drying are 1/6 and 1/9, respectively, compared to those of freeze drying (Chavez and Ledeboer, 2007). Although the spray drying environment is mild for non-bacterial powders, as shown by To and Etzel (1997), it can be severe for bacterial starter cultures. Due to this reason, the effects of drying parameters (inlet and outlet air temperatures, air flow rate, relative humidity, residence time, protective agents) on the survival and vitality of bacteria have to be understood to a considerable depth. The drying process causes damage to cell wall and cellular components, especially cytoplasmic membrane and proteins, which results in the loss of survival (Teixeira et al., 1995; Crowe et al., 1988). This cellular injury leads to cell inactivation and negatively impacts the productivity and characteristics of dried culture, and hence the cellular injury has to be minimised (Volkert et al., 2008). Protective agents such as carbohydrates, proteins,

amino acids, gums and skim milk are used to minimise the bacterial inactivation during drying. It is reported that low molecular weight carbohydrates such as sugars stabilize the membrane and protein chains of cellular macromolecules in a dry state through hydrogen bonding *in lieu* of water when the water molecules are removed through desiccation and drying (Rudolph and Crowe, 1985). Buitink et al. (2000) suggested that proteins are capable of forming relatively stable intracellular glasses, and by doing so, they can be more effective as protective materials for bacterial cultures than sugars. This is because the intracellular protein glasses were found to resist structural collapse even at 50^{o}C above the matrix glass transition temperature (T_g) while the structure of sugar glasses completely collapsed within 17-20^{o}C above the matrix T_g (Buitink et al., 2000). It is reported that the combination of different protectants (e.g. mixtures of sugar and protein) can have synergistic effect on cell viability rather than acting individually (Santivarangkna et al., 2007). Desmond et al. (2002) found that the survival of *Lactobacillus paracasei* was increased up to 10^3 fold, when 10% gum acacia was added to 10% reconstituted skim milk (Desmond et al., 2002). Hubalek (2003) and Santivarangkna et al. (2007) reported that the efficacy of protectants used depends on the strains of the bacteria being used (Hubalek, 2003; Santivarangkna et al., 2007).

It has been shown that both the water evaporation rate and the temperature of droplets containing microbial cells have a significant effect on their survival during spray drying (Etzel et al., 1996; Chen and Patel, 2007). Since it is not yet possible to quantify the changes occurring in the bacterial cells and their survival *in situ* when they are subjected to spray drying, single droplet drying is used instead. Single droplet drying provides the closest resemblance to the spray drying environment in which a single droplet is suspended in moving and conditioned air (Adhikari et al., 2000). Single droplet drying can be conducted in various ways, for example (a) a single or a stream or streams of droplets could be allowed to fall under gravity in a tower-like dryer (Alexander and King, 1985), (b) a droplet can be freely levitated using ultrasonic (Schiffter and Lee, 2007) or aerodynamic fields (Mathiak et al., 2005), or (c) a droplet can be suspended on the tip of a fine glass filament (Ranz and Marshall, 1952; Nesic and Vodnik, 1991; Yamamoto and Sano, 1992; Hecht and King, 2000; Li et al., 2006; Adhikari et al., 2007). The first two methods are not very popular as they are expensive and the heat and mass transfer rates in these environments are not close to the convective drying environment of spray drying. Li et al. (2006) investigated the inactivation kinetics of two probiotic strains (*Bifidobacterium infantis* and *Streptococcus thermophilus*) in air temperature and relative humidity range of 70-110^{o}C and 3.7-0.5%, respectively using

single droplet drying. They used skimmed milk as a suspending medium. They reported that the inactivation mainly occurred at the early stage of the drying when the evaporation rate was high (Li et al., 2006). Single droplet drying of enzymes can also be considered close to bacterial drying. In this regard, Yamamoto and Sano (1992) carried out single droplet drying experiments to measure the retention of enzymes incorporated in sugar solutions. They found that the air temperatures and droplet size significantly affected the inactivation rate while the effect of initial water content was insignificant (Yamamoto and Sano, 1992). Meerdink and Van't Riet (1995) studied the inactivation kinetics of α-amylase incorporated into maltodextrin droplets using air temperature and relative humidity in the range of 75-100°C and 4.87-1.13%, respectively. They concluded that the inactivation rate of the enzyme is more sensitive to the change in the droplet temperature rather than the rate of water evaporation (Meerdink and Van't Riet, 1995). The above studies do not offer a unanimous view whether the drying rate or the droplet temperature is the limiting factor of bacterial survival during drying.

It is necessary to find the relationship between drying kinetics (moisture history, temperature history and drying rate) and the thermal inactivation kinetics of the bacterial cultures. This can be conveniently accomplished using single droplet drying. Typical growth temperatures for *Lactococcus* are in the range of 25–35°C, above which the growth of many strains is greatly compromised. The sensitivity of *Lactococcus* to both heat and oxygen makes them ideal specimens to investigate their drying and survival kinetics as a function of drying temperature and protective agents. Furthermore, these bacteria are one of the most important cultures for many types of cheeses, fermented butter and buttermilk.

In this context, the aims of this paper are threefold. Firstly, to experimentally determine the drying kinetics (temperature history, moisture history and drying rate) and survival kinetics of a lactococcal culture during convective drying. Secondly, to investigate whether the rate of change of temperature (thermal stress) or the drying rate (dehydration stress) is the limiting factor in the survival of these bacteria. Finally, to study the effect of protective agents such as lactose, sodium caseinate and their mixture on the survival and drying kinetics of this bacterial culture.

2. Materials and methods

Lactococcus lactis subsp. *cremoris* (strain ASCC930119) was obtained from Dairy Innovation Australia and sub-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 under the same conditions. Cells were harvested by centrifugation at 2200×g for 5 minutes. This concentrated cell culture was adjusted to 10^{10} cells/ml mixing with lactose, sodium caseinate and a mixture of lactose:sodium caseinate (3:1) solutions as detailed below.

Lactose monohydrate with 99.8% purity (Sigma-Aldrich, Australia), sodium caseinate (NaCas) with protein content of 90.5% (MG2972, Murray Goulburn, Australia) were used as received.

2.1 Solution preparation

Lactose solution (10%, w/w) was prepared with deionised water at room temperature by gently stirring the solid-water mixture with a magnetic stirrer. Similarly, the sodium caseinate solution (10%, w/w) was prepared by heating the solution to 45±1°C and gently agitating the mixture with the magnetic stirrer. The lactose:sodium caseinate solution was prepared as stated above by maintaining the lactose:sodium caseinate dry ratio at 3:1 and the concentration of solids in the solution was maintained at 10% (w/w). The moisture contents in the lactose and sodium caseinate samples were predetermined and compensated for when making the solutions.

2.2 Measurement of drying kinetics

The measurements of moisture and temperature histories of the single droplets were made with a custom-built single droplet drying instrument. The details of this instrument are provided elsewhere (Adhikari et al., 2007). This instrument drew the air from a compressed air system. This air was passed through series of columns loaded with silica gel (Sigma-Aldrich, Australia) and molecular sieve (Sigma-Aldrich, Australia) to control the relative humidity (11.2% at 45°C through to 2.4% at 95°C). This air was subsequently heated to desired temperatures (45°C, 55°C, 65°C, 85°C and 95°C) using a PID controlled heating system (ECE FAST, Australia). A uniform and flat air flow pattern was created within the working section using a multi-layered wire-mesh assembly. The air velocity was maintained at 0.5 m/s throughout the experiments. The droplet holder was made from a Teflon cylinder attached to a load cell (WZ64-CW, ±0.1 mg, Sartorius, Germany). A thin glass filament (0.2

mm diameter) was inserted through a hole which was bored across the Teflon cylinder and bent on each side. Two droplets were suspended on each side of the glass filament. Each droplet (7±0.1 μL) was transferred using a 10 μL precision syringe.

2.3 Droplet mass loss measurement

Mass loss as a function of time data were measured using the above mentioned load cell and the data were recorded continuously to a computer interfaced with LABVIEW® 7.1 software (National Instruments, Texas, USA). The effect of drag on the drying data was experimentally determined and compensated for. For this purpose, a blank run without any droplets was carried out and the drift due to the drag was quantified. However, the drag values were very small or negligible in all of these experiments. The drying rates of droplets/particles were calculated from moisture content history data using equations (1), (2) and (3) for first (zero time), data points at *t* time and last (final data point), respectively:

$$\left(\frac{du}{dt}\right)_{Initial} = \frac{-3u_0 + 4u_1 - u_2}{2\Delta t} \tag{1}$$

$$\left(\frac{du}{dt}\right)_{t} = \frac{u_{t+1} - u_{t-1}}{2\Delta t} \tag{2}$$

$$\left(\frac{du}{dt}\right)_{Final} = \frac{u_{t-2} - 4u_{t-1} + 3u_{final}}{2\Delta t} \tag{3}$$

where u is the moisture content (kg water/kg solid) and t is the drying time (s).

2.4 Droplet temperature measurement

The droplet temperature was measured by suspending a droplet at the tip of a micro-thermocouple (0.3 mm, T-type, Omega Company, USA). Another micro-thermocouple with the same specification was used to measure the air temperature. The rate of change of droplet temperature (dT/dt) was calculated in the same way as the drying rate was calculated.

2.5 Measurement of cell viability

The drying experiments were stopped at pre-set times. The droplets dried to the set duration of time were immediately diluted in 1 ml peptone water (1%) (pH = 7±0.1). The live and dead cells were counted by a fluorescein diacetate (FDA) staining method (Lentini, 1993). The initial viable cell concentration was determined by using a similar droplet before it was subjected to drying. The results obtained from this method were cross-checked in some representative samples using the plate count method. The difference in estimations between these two methods was within 6%.

2.6 Isothermal water bath heating experiments

Peptone water (1%) (pH = 7±0.1) was used as the heating medium. The peptone water was first poured into a 1.5 ml Eppendorf centrifuge tube and heated in a controlled temperature water bath (Memmert Water Bath, WB-7, Germany). This way the entire Eppendorf tube-peptone system was allowed to reach the set temperature before the culture mass was added. Subsequently, same cell culture concentration (10^{10} cells/ml) was added to heated peptone water which was further heated for about 10 minutes at the set temperature. This way the cells were brought to the set isothermal temperature fairly quickly. The temperature profile within the Eppendorf was monitored using above mentioned temperature measuring system. This allowed monitoring of the temperature profile of the content within the Eppendorf. The come up time (the time required for the content of the Eppendorf (pepton water + bacterial culture mass) to reach the set water bath temperature after the bacterial culture was introduced) was measured at 45^{o}C, 65^{o}C and 95^{o}C. For this purpose, the microthermocople was inserted within the Eppendorf by boring a hole on its cap (lid). The survival versus time data were measured in each temperature setting once the bacterial cultures reached the set isothermal drying temperature (first by measuring the come up time).

2.7 Statistical analysis

Statistical analysis was performed using ANOVA. The mean values and standard deviations 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 15 software (Minitab, Australia).

3. Results and discussion

3.1 The drying kinetics

Figure 1 (panels a and b) shows the temperature and moisture content histories of the bacterial culture droplets at different air temperatures (45°C, 55°C, 65°C, 85°C and 95°C), respectively. As can be seen from Figure 1(a) the droplet temperature rose up rapidly after the start of drying (heating up period) and then levelled off briefly when the drop temperature approached the wet-bulb temperature (the wet-bulb temperatures were 22°C, 25°C, 28.5°C, 34°C and 36.5°C for air temperatures of 45°C, 55°C, 65°C, 85°C and 95°C, respectively) and later increased to the dry-bulb temperature. As can be seen in this figure, the drop temperature approached the dry-bulb temperature rapidly at higher dry bulb or drying air temperatures but somewhat gradually when lower dry-bulb temperatures were used. At the same time, moisture content of the droplet decreased continuously until the change in moisture content between two consecutive time periods (10 seconds apart) became negligibly small at the set air temperature and relative humidity (Figure 1b).

Figure 2 (panels a and b) shows the rates of change in droplet temperature and moisture content, respectively. As can be seen from Figure 2(a) while the droplet temperature remains in the vicinity of wet-bulb temperature, the increase in the dT/dt is very slow and this slow increase in the dT/dt continues for a longer time when lower dry-bulb temperatures are used. When higher drying temperatures are used, the increase in the magnitude of dT/dt occurs fairly quickly. Figure 2(b) shows the drying rate (-du/dt) continuously decreases over time at any dry-bulb temperature. It can also be seen from this figure that in the early stage of drying, the higher the air temperature, the greater the rate of moisture evaporation.

Figure 3(a) presents the drying rate (du/dt) versus droplet temperature data of bacterial cultures in the absence of protective agents (air temperature 45^{o}C-95^{o}C). One of the interesting features of these plots is that the drying rate of the droplet does not seem to be constant as a function of droplet temperature. All of these plots exhibit three distinct regimes in drying rates. The drying rates are high in the beginning and their fall or decline is very mild. There is a distinct regime where the decrease in the drying rate is slow, smooth and continuous in the vicinity of the wet bulb temperature and in the early stage where the droplet temperature starts to rise. Thirdly, there is sharp fall in drying rate as the droplet temperatures approach closer to the set air temperature. The difficulty in outward diffusion of water from

interior to the surface of the droplets towards this third stage gives rise to the lowest drying rates accompanied by their rapid fall as the temperature rises towards the dry bulb temperature. As expected, the drying rates are consistently higher at 95^{o}C compared to other lower dry bulb temperatures.

The drying rate versus droplet temperature plots of bacterial cultures in the presence of protectants at drying air temperature of 65°C are presented in figure 3(b). As can be seen from these plots that the overall dehydration rate of the droplets containing protectants decreases compared to when they are absent. When the protective agents are added, the total solid content in the droplet increases (consequently the water content decreases). The increased solid matrix within the droplet reduces the evaporation rate. This is expected as the diffusion coefficient of water decreases significantly when the solid content increases and the increased solid mass offers a greater resistance to the outward diffusion of the moisture from the interior (Adhikari et al., 2002). Furthermore, solids such as lactose and sodium caseinate interact differently with water and allow release of water from their matrix quite differently. It can also be seen from Figure 3(b) that the water evaporation rate in droplets containing lactose is the lowest. However, the lactose and sodium caseinate interact synergistically to facilitate faster evaporation of water from the lactose-sodium caseinate matrix. This can be seen from higher evaporation rates in droplets containing lactose:NaCas (3:1) than in droplets containing sodium caseinate alone. These results corroborate earlier finding that small molecular weight sugars occupy the free volume created by removal of water in high molecular weight biopolymers such as proteins and carbohydrates (Mauerer and Lee, 2006). The increased occupation of free volume would lead to greater expelling of the water ultimately resulting into higher water evaporation rates.

Figure 3(c) shows the rates of change in droplet temperature (dT/dt) of bacterial cultures in the presence of protectants. The dT/dt values at drying air temperature of 65^{o}C are used here for illustration. It can be seen from this figure that the maximum change in dT/dt occurs in droplets containing no protective agents (approx. 0.23°C/s at 41.2°C). The addition of lactose, sodium caseinate and lactose:sodium caseinate (3:1) as protective agents decreases the peak dT/dt values to 0.14°C/s, 0.10°C/s and 0.15°C/s, respectively. This lowering of the magnitude of dT/dt may be one of the key mechanisms through which these protective agents help to protect the bacteria during drying process (refer to „Survival kinetics" section for more details). Furthermore the peak dT/dt values, in the presence of protectants, occur at higher

temperatures. This means that the bacterial cells in the presence of protectant only get exposed to higher dT/dt when the droplets are already dried to a greater extent. Both of which are conducive for protection of bacterial cells during drying. A similar trend was observed at the drying air temperature of 85 °C at which the effects of protective agents were studied (data not shown). At high drying temperatures (such as 85^{o}C) the dT/dt values were higher than their corresponding values at lower temperatures (such as 65^{o}C) which indicate that the droplet temperature approaches the higher drying temperature faster. These trends in increase in droplet temperature could explain why drying temperature is a crucial determinant of bacterial survival or death.

The characteristic drying rate curves (rate of change of moisture content (du/dt) versus moisture content (u)) for this bacterial cultures (in the absence of protectant solids) at five different drying air temperatures are presented Figure 4. It is interesting to note that even when the initial moisture content of the droplet was considerably high (18.5 kg water/kg solid), each individual rate curve showed the absence of the constant drying rate. As can be seen from this figure, the rate of moisture content loss was very high at all temperatures in the earlier stage of drying (when the moisture content is high), but decreased as the moisture content fell. Below the moisture content of 2 kg water/kg solid, all the curves sloped steeply, rapidly approaching a zero rate of moisture loss. This can be due to approaching of the equilibrium moisture content of the bacterial cellular material at the prevailing drying air condition.

The experimental droplet temperature data versus moisture content are presented in Figure 5. This figure shows that the droplet temperatures did not maintain constant values even when they were close to their respective wet-bulb temperatures. This corroborates with Figure 4 that there was absence of a noticeable constant rate regime. This indicates that the bacterial biomass becomes sensitive to drying temperature even at very early stage of drying further explaining that the survival of bacteria is very sensitive to drying temperature.

3.2 The survival kinetics

The survival scenario of this bacterial culture during single droplet drying at different air temperatures of 45°C, 55°C, 65°C, 85°C and 95°C is presented in figure 6(a). This graph shows that the survival is affected by drying air temperature used and that the survival declines with the increase in the drying time. For example, the survival of bacterial culture

dried for 15 minutes was 88.1% at 45°C. However, when the air temperature was increased by 10°C (to 55°C) and the drying was conducted for 9 minutes, only 55.3% of the bacterial cells survived. The survival of the bacterial cells reduced to less than 10% when the cells were dried at 95°C for 6 minutes. At higher air temperatures (65°C or above) the survival of bacterial cultures dropped significantly ($p<0.05$) within 2 minutes of drying.

In order to compare the survival of these lactococcii in a convective (single droplet) drying and in an isothermal water bath heating system, the survival versus time data of the bacterial culture were measured using a water bath maintained at 45°C, 65°C and 95°C (Figure 6b). Figure 6(c) presents the temperature profile during the isothermal water bath heating process (45±0.4°C, 65±0.3°C and 95±0.5°C). As can be seen from this figure, the addition of the bacterial starter culture cell mass to the heated peptone water rapidly lowered the temperature of the mixture (peptone+bacterial cells) approximately by 10°C. However, the temperature of the entire content within the Eppendorf climbed rapidly back to the set temperature within 75, 60 and 45 seconds at 45°C, 65°C and 95°C, respectively. These temperature profile data show that the come up time is quite significant and hence we enumerated the survival of the bacterial cells at the end of the come up time at each set water bath temperature. The survival of the bacterial cells at the end of come up time was taken as 100% when determining the inactivation kinetics parameters.

Figure 6(b) shows that the survival of bacterial cells within the first minute of heating was reduced significantly ($p<0.05$) to 86.1%, 75.5% and 56.2% compared to single droplet drying (99.6%, 89.6% and 77.3%) at 45°C, 65°C and 95°C, respectively. Heating the bacterial cells for 6 minutes decreased the survival of bacterial cells to 34.3%, 19.5% and 4.4% at 45°C, 65°C and 95°C, respectively. The corresponding values of bacterial cultures survival during the convective (single droplet) drying were 97.3%, 18.8% and 5.7%, respectively. These data show that the extent of survival of *lactococcii* during isothermal water bath heating is quite different compared to convective (single droplet) drying at identical heating medium temperature. These significantly higher death rates during isothermal water bath heating compared to those occurring in convective air drying can be attributed to two factors. Firstly, the droplets in the latter case experience evaporative cooling and their temperature reaches the set air temperature only towards the end of drying. Secondly, the moist heat experienced by the cells in the former case may be more lethal and is more effective in inactivating the cells than the less moist heat in the latter case.

The survival of bacterial starter cultures as a function of moisture content showed different patterns at different temperatures during drying (Figure 7a). As can be seen from this figure, at low air temperature (45°C) the reduction in bacterial survival seems to depend on drying (exposure) time rather than the loss in the moisture content. Even when the moisture content is below 1.5 kg water/kg solid, the survival is still high (87.8%). This is because the bacterial cells are reasonably comfortable at the prevailing droplet temperature. The longer drying (exposure) time increased the death rate albeit only slightly. At higher air temperatures (≥ 65°C) the reduction in the droplet moisture content resulted into reduction of survival significantly ($p<0.05$).

The effect of protectants on the survival of bacterial cultures as a function of moisture content at air temperature of 65°C is presented in figure 7(b). This figure shows that towards the end of the drying process only 15.9% of initial bacterial cells survived the drying process in the absence of protectants. The percentage survival of the bacterial cells improved quite significantly ($p<0.05$) when the lactose and sodium caseinate were used (individually) as protectant solids compared to the survival of the bacterial cultures when these protectant solids were not used. The survival of the bacterial cells was 68.6% and 64.8% in the presence of lactose and sodium caseinate, respectively. These survival data suggest that the protective effect of lactose and sodium caseinate seems very close to each other as these two survival data are not significantly different statistically ($p<0.05$).

It has to be noted here that the mechanisms through which the low molecular weight sugars such as lactose and high molecular weight macromolecules such as proteins effectuate the protective effect are quite different. The protective efficacy of lactose can be attributed to its readily hydrogen bonding propensity (direct interaction) (Oldenhof et al., 2005). The flexible-sieve (or fisherman"s net) like structure of peptidoglycan in the cell wall of Gram-positive lactic acid bacteria allows molecular size of 25 kDa trough the net in the normal and unscratched state (Delcour et al., 1999). This means that lactose molecule could easily diffuse through the sieve opening to reach the plasma membrane. This allows the lactose molecules to form hydrogen bonds with polar groups of peptidoglycan, lipoteichoic acid, teichoic acid and S proteins. It is also possible for lactose to form hydrogen bonds with polar head groups of phospholipids of plasma membrane. The bonding of lactose to cell wall and plasma membrane components through hydrogen bonds helps resisting the compressive pressure exerted to the cells during the evaporation of water from the solution as well as from

within the cells. This so called hydration force extension is proposed as one of the key mechanisms through which low molecular weight sugars like lactose provides protection to the bacterial cells during drying (Oldenhoff et al., 2005; Santivarangkna et al., 2007; Buitink et al., 2000). On the other hand, macromolecules such as sodium caseinate may not pass through the cell wall network. This means that the proteins will only be able to form non-interacting (no hydrogen bond formation) osmotically inactive bulking compounds causing spacing amongst cells and not allowing their cell wall to come closer and fuse (Oldenhof et al., 2005). Macromolecules such as sodium caseinate also facilitate formation of structurally stable glassy network at much higher moisture content compared to low molecular weight sugars such as lactose (Prestrelski et al., 1993).

The mixture of lactose:sodium caseinate (3:1) improved the survival to 81.8% which was statistically significant compared to survival provided by these solids individually ($p<0.05$). It means the protectant solids comprising both sugars and proteins (in this case lactose and sodium caseinate) provide better survival than when they are used individually. The efficacy of sugar-protein mixture can be attributed to the synergistic effect coming from both hydrogen bonding (from sugar) and no-interacting bulking (from protein) mechanisms. This is in agreement with various reports that the mixed protectant systems such as carbohydrates and proteins can have a synergic effect on cell viability and preventing cellular injury during dehydration compared to when they act individually (Santivarangkna et al., 2007; Buitink et al., 2000).

As can be seen in figure 8(a), at the drying temperature of 45°C the plots of the rate of change of droplet temperature (dT/dt) and the rate of survival (dS/dt) as a function of droplet temperature have similar trends. When the droplet temperatures was 30.5°C the dT/dt was maximum and the dS/dt reached the peak value with very minor offset of the peak points of these two variables. This suggests that at low drying temperature when the evaporation flux from the droplet is very low, the outward moisture diffusion from the bacterial cell is benign and the cellular structure is not too severely altered. This means that the death caused by the rate of moisture loss is not as dominant as the death caused by the rate of rise in temperature.

Figure 8(b) shows that at higher temperatures, for example at 95°C, highest value in the cellular death rate was observed at the droplet temperature of 44.9°C (at 2 minutes of drying); however, the highest value of dT/dt (0.31°C/s) occurred at the droplet temperature of 70.5°C at 3 minutes of drying. A similar trend can be observed while dying the bacterial cells

at 65°C. At this drying temperature, the peak value in dT/dt occurred well after the peak in the death rate (dS/dt) occurred (Figure 8b). These facts suggest that the rate of moisture evaporation would be contributing to the death of the bacterial cultures significantly. As the moisture evaporation flux and rate of rise in temperature are very high in droplets dried at elevated temperature (such as 95°C), it is expected that the cellular structure, especially that of proteins and phospholipids is severely affected with such high evaporation fluxes and the rapid rise in temperatures within the cell beyond their comfort zone.

As mentioned earlier, at higher air temperatures (at and above 65°C) both the water evaporation rate and the rise in droplet temperature affect the bacterial death; however, the high moisture evaporation rate in the early stage of drying is dominant in killing the bacterial cells at high temperatures.

Figure 9 (panels a and b) shows the rate of change in droplet temperature and survival of bacterial culture with protective agents as a function of droplet temperature when the drying air temperature was set at 65°C. It can be seen from this figure that the maximum dT/dt value is reduced approximately by half in the presence of protectant solids. In addition, the peak dT/dt values occurred at higher droplet temperatures (Fig. 9a). Both panels (a and b) in figure 9 show that in the presence of protectants, the maximum rates of change in survival and droplet temperature occurred at the same droplet temperature or droplet temperatures which are very close to each other. This suggests that in the presence of protectants the droplet temperature (not the evaporation rate) becomes more dominant factor causing the death of bacteria.

Figure 9(b) shows that at drying temperature of 65°C, the maximum death rate of bacterial cells in the absence of protectants occurred at droplet temperature of 31.7°C. However, in the presence of 10% (w/w) lactose, sodium caseinate and lactose:sodium caseinate (3:1) as protectants, the maximum change in survival (dS/dt) occurred at droplet temperatures of 44.1°C, 44.3°C and 48.6°C, respectively. Furthermore, the upper limit of bacterial death (or rate of death) became much lower in the presence of the protective agents. As stated previously, the low molecular weight sugars stabilize the cell wall and plasma membrane of bacterial cells preventing the denaturation of proteins and fusion of phospholipids (when the amount of cellular water continuously decreases) through the formation of hydrogen bonds *in leu* of water (Santivarangkna et al., 2006; Leslie et al., 1995; Ananta et al., 2005; Roos and Karel, 1991). Furthermore, addition of protective agents such

as low molecular sugars and proteins can help raise the viscosity of the matrix as the drying progresses. However, as the residual moisture content of these droplets is of the order of 0.15 kg water/kg solid, it is unlikely that these particles had attained a viscosity of 10^{12}-10^{13} (kPa/s) commonly prevailing in glassy solids (Roos and Karel, 1991) and became glassy under the prevailing drying condition. The molecular mobility (diffusion) of water within the particle matrix at this level is greatly slowed down but still significant rather than being arrested. Hence, the preferential hydrogen bonding between the lactose with various components in the bacterial cell wall and the plasma membrane combined with mainly non-interacting type hydration force expansion (spacing the cells) through proteins are most likely mechanisms with which these protectant solids have managed to improve the survival of the bacterial cells during these drying experiments. Furthermore, it is also possible that both the lactose and sodium caseinate can shield these desiccated bacterial cells from coming in contact with oxygen. It is known that lactococcii are facultative bacteria and that the oxygen is toxic to them and that the oxidative stress is exacerbated by elevated temperatures (Cesselin et al., 2009). For oxygen-sensitive bacteria like lactococcii, shielding of partially or fully dehydrated cells from oxygen and preventing especially the phospholipids in forming reactive oxygen species probably is the another mechanism through which these protectant solids help better survival of bacterial cells during drying.

3.3 The inactivation kinetics

The inactivation kinetics of the bacteria can be described by a „nth“ order reaction kinetics model which is popularly used in solid state chemical reactions (Nzihou and Adhikari, 2004):

$$\frac{dS}{dt} = - K_d \, S^n \qquad (4)$$

where, S is the survival of the bacteria (%), K_d is inactivation rate constant (1/s) and *n* is the reaction order (dimensionless). Equation (4) can be integrated using initial survival of bacteria (S_0 = 100%) as given by equation (5). In this analysis equation (5) is fitted by minimising the chi-square value in order to determine K_d and *n* values for each temperature represented by equation (6). We wish to emphasize here that the temperature we mentioned is the final temperature attained by the droplets. In this way we avoided using the temperature of the drying air. As the droplet profiles were almost flat during the last 60 seconds of drying

in each set of air temperature used, we averaged the droplet temperatures recorded for the final 60 seconds and used the averaged value as the final temperature attained by the droplets. Although the droplet temperature varies from room temperature to the set drying temperature, only one inactivation rate constant (K_d) was found to be sufficient in solving Equation (5). This supports the use of final particle (droplet) temperature (corresponding to each drying temperature) to determine the K_d. This avoids the complication of requiring K_d values for each droplet (particle) temperature along the temperature history. Furthermore, this simplification allows calculation of temperature dependence of K_d using the maximum attainable droplet (particle) temperatures as shown in Equation (7). The Solver™ program of Microsoft Excel™ (Microsoft® Office Excel 2003) was used for this purpose.

$$\left.\begin{aligned} S_t &= \left(S_0^{1-n} + (n-1)\,K_d t\right)^{1/(1-n)} && n \neq 1 \\ S_t &= S_0\; e^{-K_d t} && n = 1 \end{aligned}\right\} \tag{5}$$

$$\chi^2 = \frac{1}{N-(p-1)} \sum_{i=1}^{N} \frac{\left(S_i^{exp} - S_i^{pred}\right)^2}{S_i^{exp}} \tag{6}$$

where, "exp" refers to experimental % survival data and "pred" denotes predicted % survival data obtained through the model prediction. N is the total number of observations and p is the number of unknown parameters or constants. Temperature dependence of the inactivation rate constant (K_d) was subsequently determined by using equation (7) below:

$$K_d = K_{d\,Ref}\left[1 - \frac{E_a}{R}\left(\frac{1}{T} - \frac{1}{T_{Ref}}\right)\right] \tag{7}$$

where, $K_{d\ Ref}$ is the inactivation rate constant (1/s) at a reference temperature, E_a is inactivation energy (J/mol), R is universal gas constant (J/mol.K), T is the maximum attainable droplet temperature (K) as mentioned above. T_{Ref} is the reference droplet temperature (maximum droplet temperature attained at set air temperature of 45°C) which was 318.15 K.

The experimental and the model predictions of survival kinetics (without protectant solids) for the entire single droplet drying experiments are presented in figure 6(a). The model predicted and the experimental bacterial survival values are reasonably close and are within 1.23% (minimum) and 8.22% (maximum) experimental error. The average absolute

error was 4.5%. The reaction order (n) at each of the maximum droplet temperatures was very close to 1 [n =1 in equation (5)] indicating that the inactivation process followed the first order kinetics. At 65°C and above, the survival of the bacterial cultures did not change significantly within the first minute of drying ($p<0.05$) at all of the set drying air temperatures perhaps due to the fact that the droplet temperature increased only marginally and very slowly within this time and remained in the vicinity of the wet-bulb temperature. Since the maximum droplet temperatures of bacterial cultures within this time frame (in all set air temperatures) was 36.5°C, the bacterial cultures were in their comfort zone. Generally, the temperature dependence of the inactivation rate constant (K_d) of bacterial survival is predicted using an Arrhenius type of model (Li et al., 2006). However, our experimental K_d values were best represented by an inverse linear function of droplet temperature expressed in Kelvin (Eq. 7). The inactivation energy (E_a) was found to be 181.3 kJ/mol.

In order to compare the inactivation behaviour of these lactococcii in a convective air drying and in an isothermal water bath heating process, we measured the survival of the bacterial culture using a water bath maintained at 45°C, 65°C and 95°C. The survivals versus time data measured at these temperatures are presented in Figure 6(b). In order to determine the inactivation rate constant (K_d) and reaction order (n) values for each temperature, equation (5) was fitted using non-linear least square method. Furthermore, equation (7) was used to determine the temperature dependence of the inactivation rate constant (K_d) as the same way it was used to fit the survival data of this bacterial culture during convective (single droplet) drying.

Figure 6(b) shows the experimental and the model fitted data of survival kinetics obtained from isothermal water bath heating experiments. The average absolute error between the model predicted and experimental bacterial survival was 6% which varied from 2.6% (minimum) to 8.6% (maximum). The inactivation behaviour of these lactococcii during isothermal water-bath heating was followed the first-order kinetics as well. Furthermore, at 2 minutes of heating, the survival of bacterial cells was 66.7%, 54.9%, and 31.2% at 45°C, 65°C and 95°C, respectively. The corresponding values of the survival during convective (single droplet) drying process were 99.4%, 67.2% and 41.8%, respectively. Similarly, at 6 minutes of heating, the survival of bacterial cultures was 34.3%, 19.5%, and 4.4% at 45°C, 65°C and 95°C, respectively. The corresponding values of the survival during convective (single droplet) drying process were 97.3%, 18.8% and 5.7%, respectively. When the survival

was measured at the first minute, the survival of the bacterial cells during convective drying was greater than 75% even at 95°C; however, the survival of the bacterial cells during water-bath heating was 86.1%, 75.5% and 56.2% at 45°C, 65°C and 95°C, respectively. These data show that the extent of survival these lactococcii during isothermal water bath heating is significantly different compared to their survival during convective (single droplet) drying at the same medium temperature ($p<0.05$). The experimental K_d values obtained during water bath heating experiments were also fitted well by an inverse linear function of droplet temperature (Eq. 7) and the inactivation energy (E_a) was found to be 16.8 kJ/mol.

Table 1 presents the inactivation rate constants (K_d values) obtained at 45°C, 65°C and 95°C obtained from convective (single droplet) drying and isothermal heating experiments. These K_d data show that the rate of inactiation in convective air drying environment at each set medium temperature is smaller than the rate of inactivation at the corresponding medium temperature in isothermal water bath heating environment. The ratio of the inactivation rate constants (K_d value in single droplet drying/K_d value in isothermal water bath heating) were increased from 0.051 to 0.951 and 0.751 at medium temperature of 45°C, 65°C and 95°C, respectively. Similarly, the inactivaiton energies of these process show that the enactivation energy of the single droplet drying is about 10.8 times that of isothermal water bath heating. This further implies that much higher energy is needed to inactivate these bacterial cells during convective single droplet drying environment compared to the energy required during the corresponding water bath heating environment.

4. Conclusions

In this study, the drying and survival kinetics of aqueous *Lactococcus lactis* suspensions were investigated in the absence and presence of protective agents. Single droplet drying experiments were used to investigate the drying and survival kinetics during convective drying. The results showed that survival of these cultures was affected differently by the rate of moisture evaporation and the rate of rise in droplet temperature. At and above the droplet temperature of 65°C, it was found that the bacterial death occurred due to both the dehydration and thermal stresses. At lower droplet temperatures than 55°C the rate of change of droplet temperature and drying time (exposure time) had much stronger effect on the bacterial death. The presence of lactose and sodium caseinate as protectant solids was found

to significantly improve the survival of bacteria during drying. Our experimental results showed that the inactivation of the *Lactococcus lactis* bacteria followed first-order reaction kinetics during convective single droplet drying as well as during isothermal water bath heating at the same temperature. The temperature dependence of the inactivation rate constant (K_d) for these bacteria was best predicted by an inverse linear function of droplet temperature expressed in absolute temperature (K) for both the convective single droplet drying and isothermal water bath heating experiments. The inactivation energy in convective single droplet drying environment was found to be 10.8 times higher compared to that in the isothermal water bath heating environment within the medium temperature of 45-95^{o}C. Hence, these bacteria are found to be very sensitive to heating in water than drying in convective environment.

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NOMENCLATURE

E_a	Deactivation energy (J/mol)
K_d	Inactivation rate constant (1/s)
$K_{d\ ref}$	Reference inactivation rate constant (1/s)
n	Reaction order (dimensionless)
R	Universal gas constant (J/mol.K)
S	Survival of the bacteria (%)
S_0	Initial survival of the bacteria before drying (%)
t	Time (s)
T	Droplet temperature (K)
T_{Ref}	Reference temperature (K)
u	Moisture content (kg water/kg solid)

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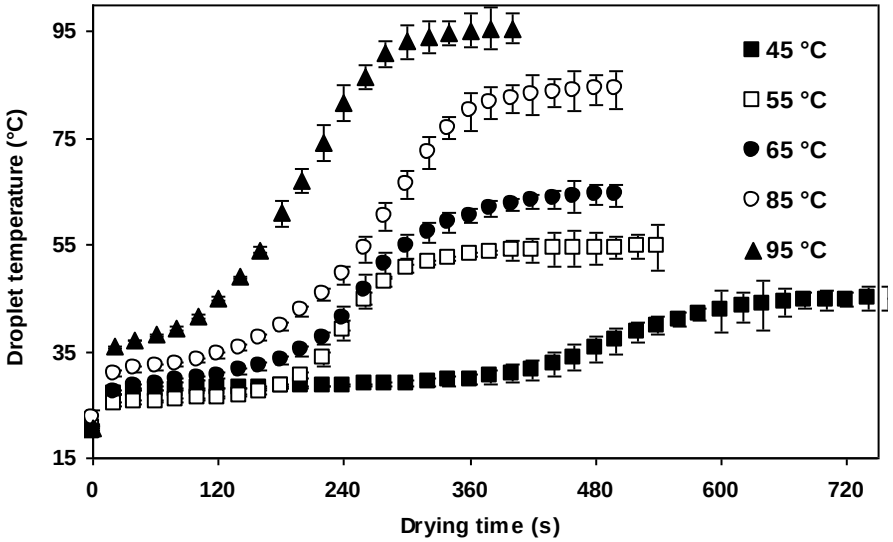
Fig. 8. The rates of change of survival and droplet temperature of *L. lactis* as a function of droplet temperature during single droplet drying in the absence of protectants at 45°C (a) and 65°C, 95°C (b). (dS/dt (■,▲) and dT/dt (□,Δ)).

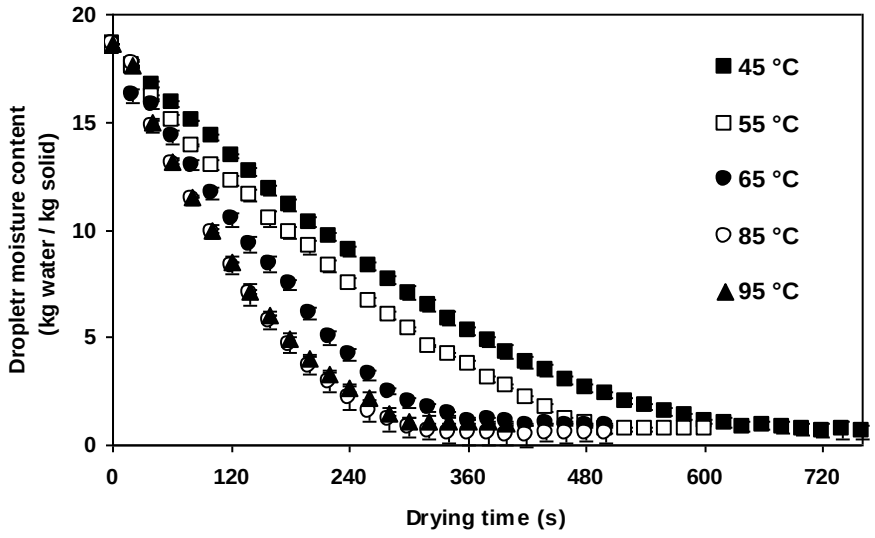
Fig. 9. The rates of change in droplet temperature (a) and survival (b) of *L. lactis* as a function of droplet temperature during single droplet drying at air temperature of 65°C.

Table 1. The inactivation rate constants of *L. lactis* at different temperatures during single droplet drying ($K_{d\,S}$) **and** isothermal heating ($K_{d\,Iso}$) processes

	45°C		**65°C**		**95°C**	
	K_d (1/s)	**$K_{d\,S}$ / $K_{d\,Iso}$**	**K_d (1/s)**	**$K_{d\,S}$ / $K_{d\,Iso}$**	**K_d (1/s)**	**$K_{d\,S}$ / $K_{d\,Iso}$**
Single droplet drying	0.00016	0.0510	0.004081	0.951	0.005872	0.751
Isothermal heating	0.003136		0.004291		0.007818	

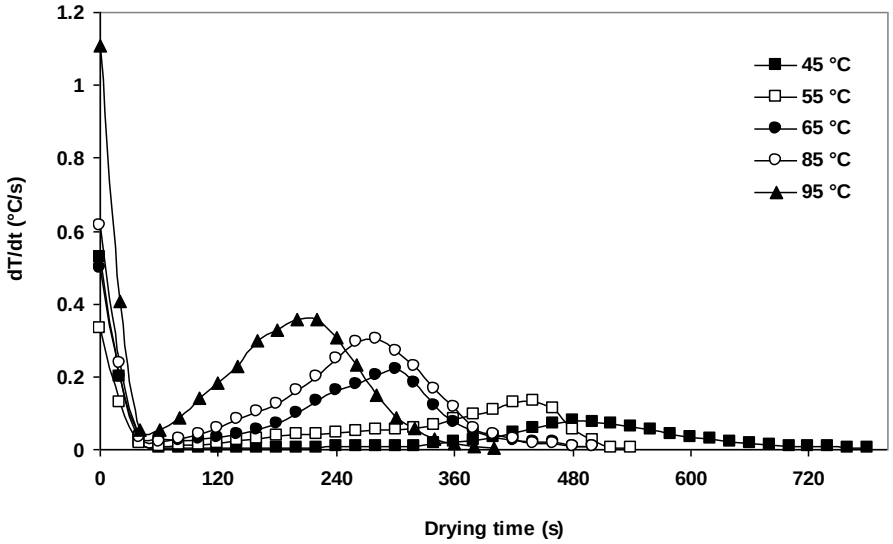


(a)

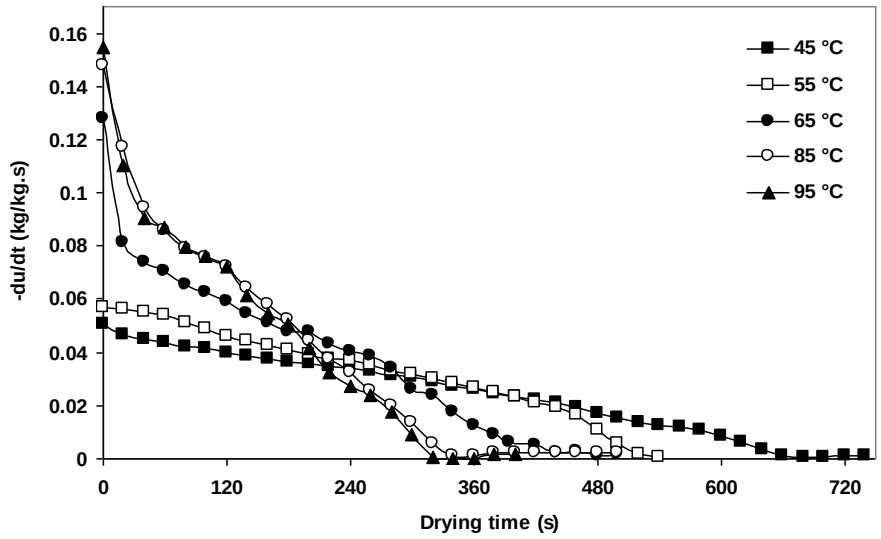


(b)

Fig 1 Temperature and moisture content histories of single droplets of *L. lactis* culture at different drying air temperatures.

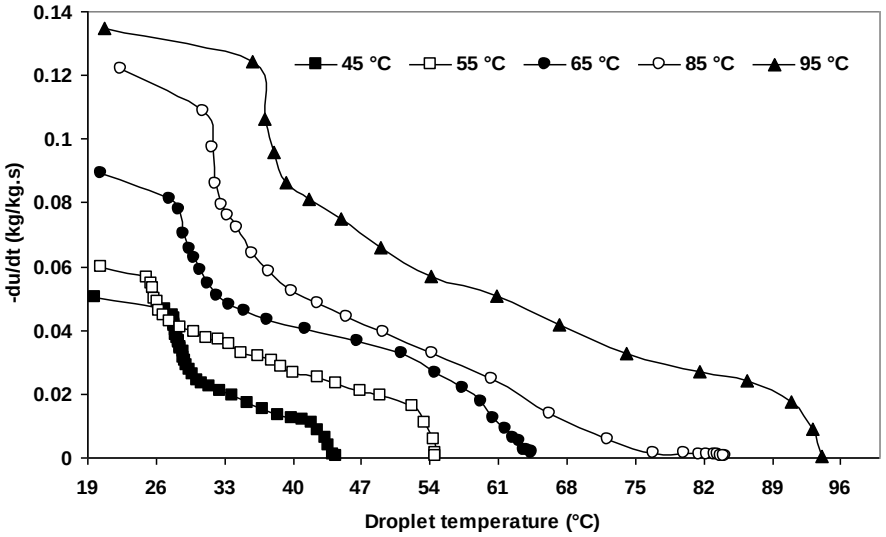


(a)

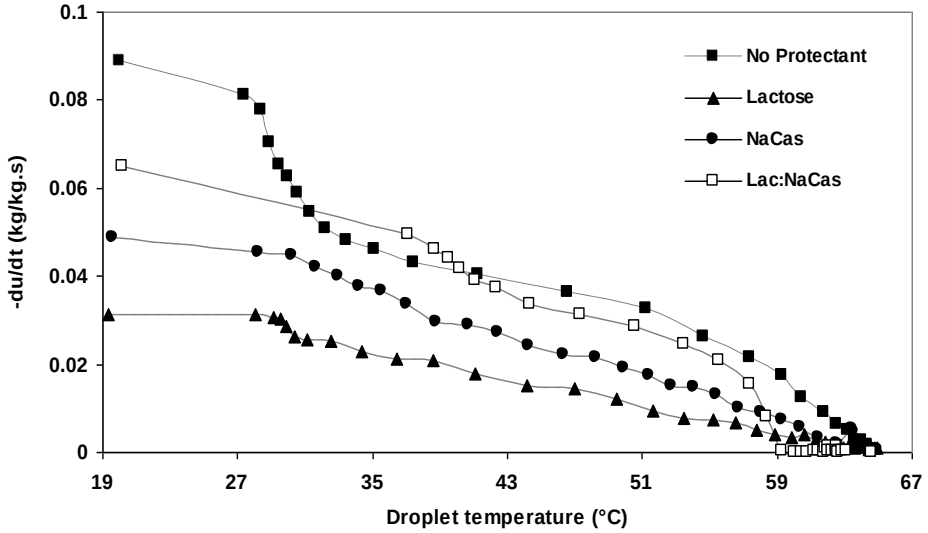


(b)

Fig. 2 The rates of change in the droplet temperature, dT/dt (a) and the moisture content, du/dt (b) during drying of *L. lactis* culture droplets at different air temperatures.


45 °C
55 °C
65 °C
85 °C
95 °C
-du/dt (kg/kg.s)
Droplet temperature (°C)

(a)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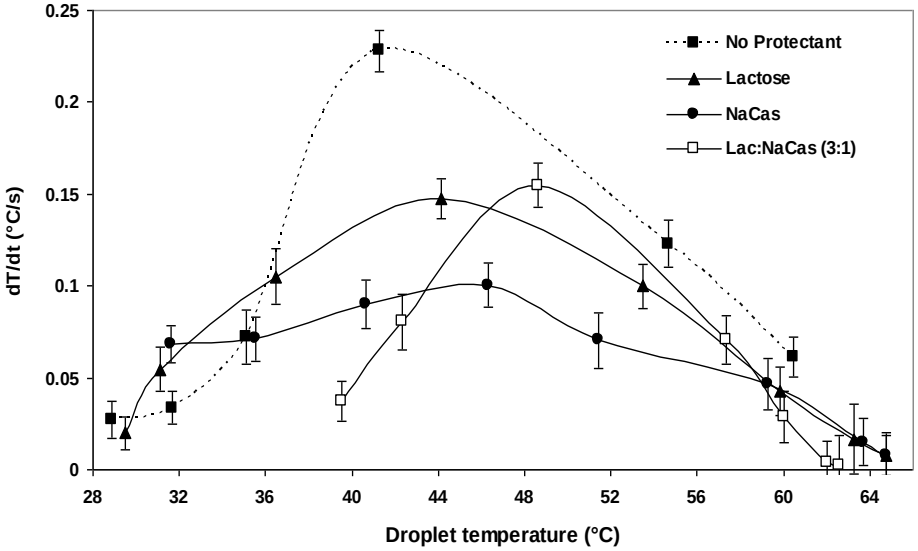
Lactose
NaCas
Lac:NaCas
-du/dt (kg/kg.s)
Droplet temperature (°C)

(b)


No Protectant
Lactose
NaCas
Lac:NaCas (3:1)
dT/dt (°C/s)
Droplet temperature (°C)

(c)

Fig. 3 Drying rates (du/dt) versus droplet temperatures of *L. lactis* at different air temperatures in the absence of protective agents (a). The drying rates versus droplet temperature of the same bacteria in the presence of protectant solids at drying air temperature of 65°C (b) .The rate of change in the droplet temperature (dT/dt) in the presence of protective agents at drying air temperature of 65°C (c).

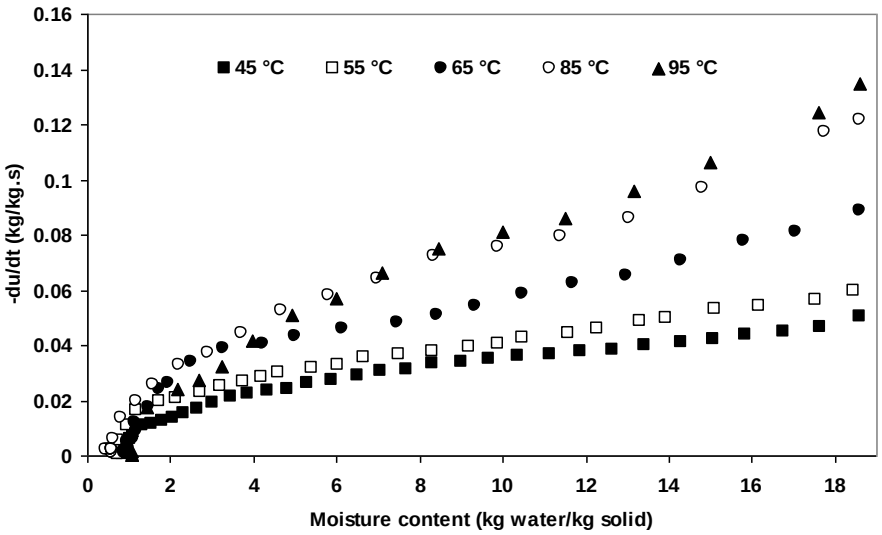


Fig. 4 Drying rate versus moisture content of *L. lactis* culture droplets at different drying air temperatures.

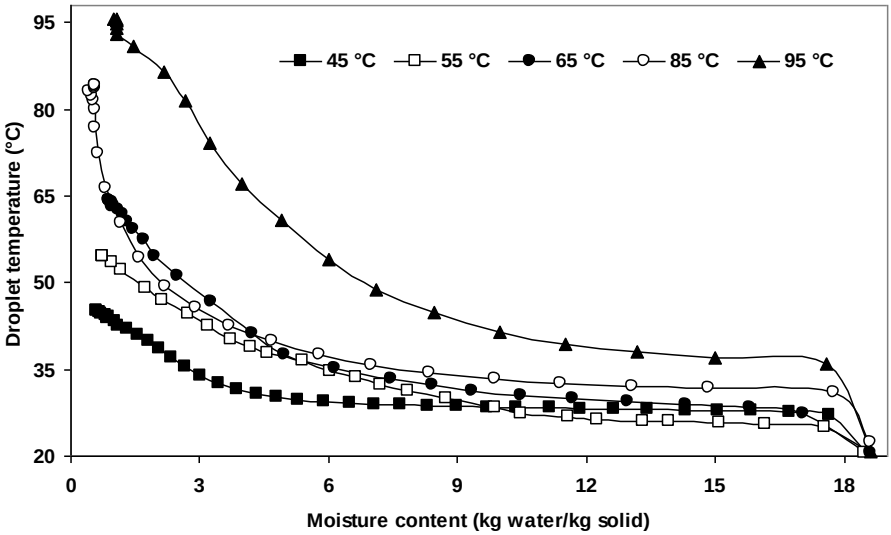
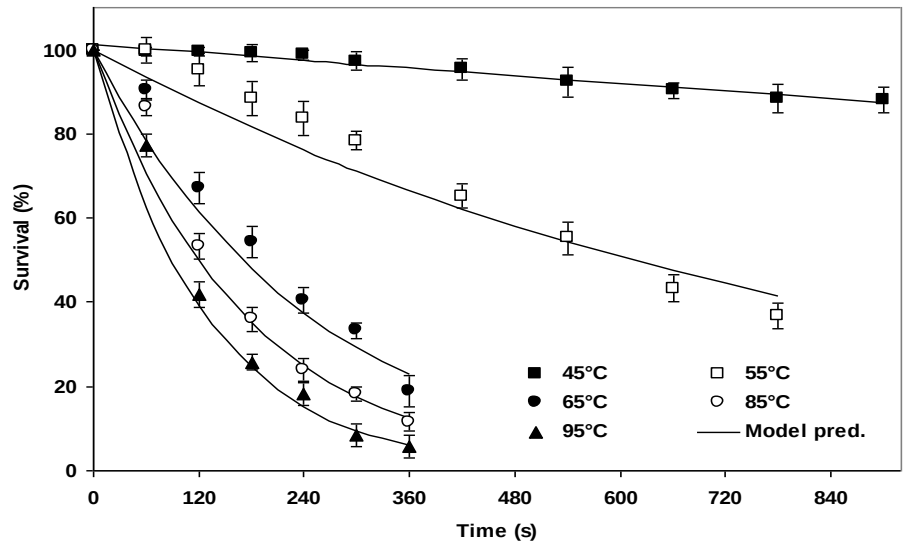
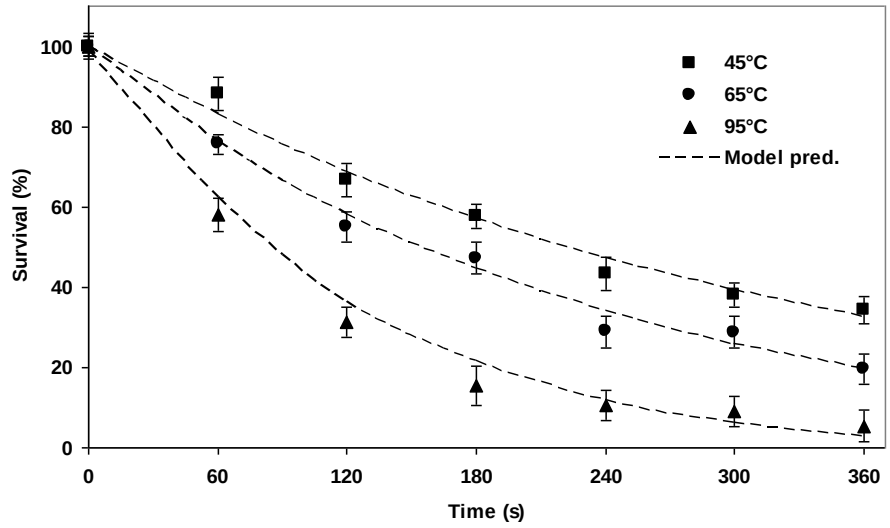


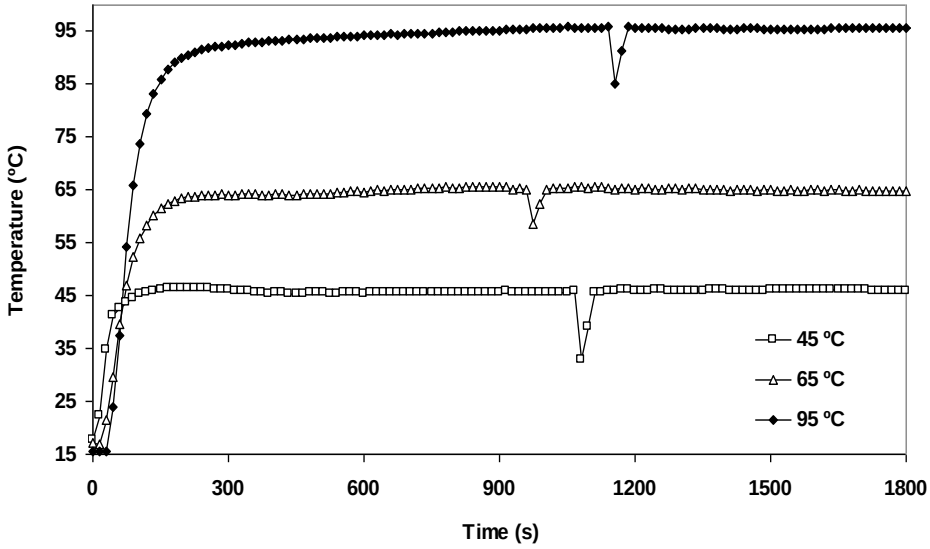
Fig. 5 Droplet temperature versus moisture content of *L. lactis* culture droplets at different drying air temperatures.


Survival (%)
Time (s)
45°C
65°C
95°C
55°C
85°C
Model pred.

(a)

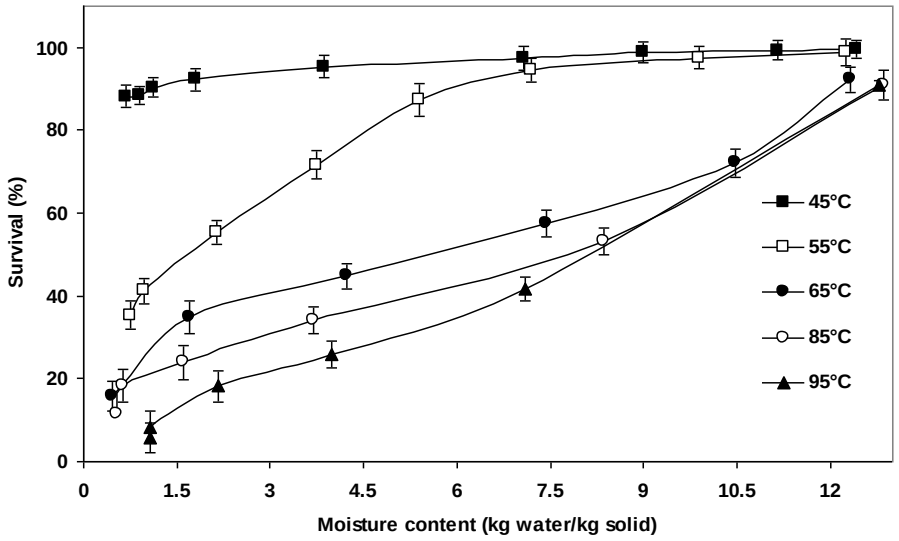

Survival (%)
Time (s)
45°C
65°C
95°C
Model pred.

(b)

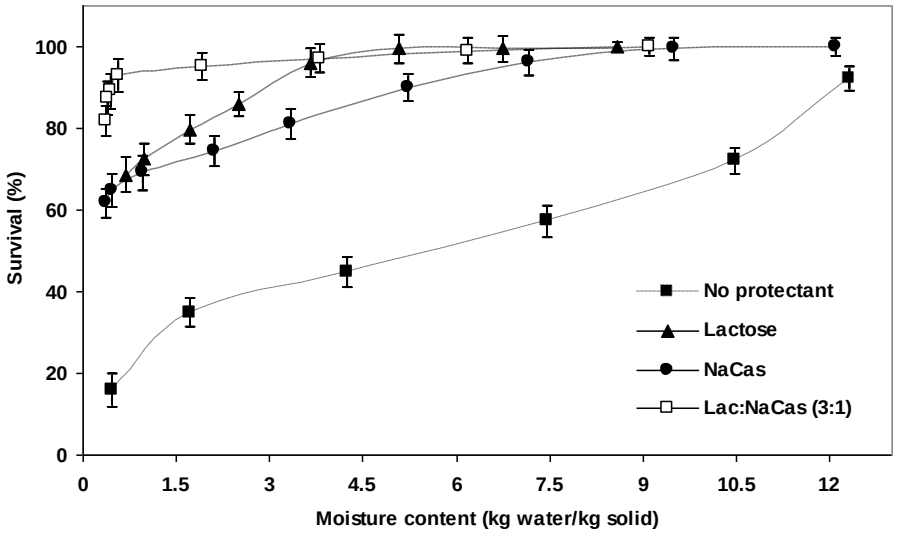

Temperature (°C)
Time (s)
45 °C
65 °C
95 °C

(c)

Fig. 6 Survival kinetics of *L. lactis* during single droplet drying at different drying air temperatures in the absence of protectants (a). Isothermal water bath heating of the *L. lactis* cultures at different temperatures (b). The temperature profile of the peptone water and the bacterial cells within the Eppendorf (c). (Model prediction lines (solid/dotted lines) obtained using Eq. (7)).

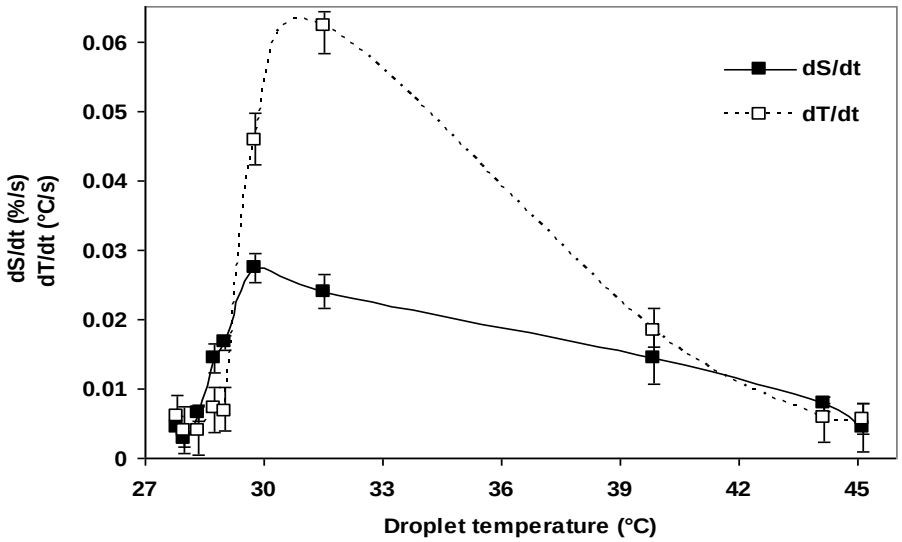


(a)

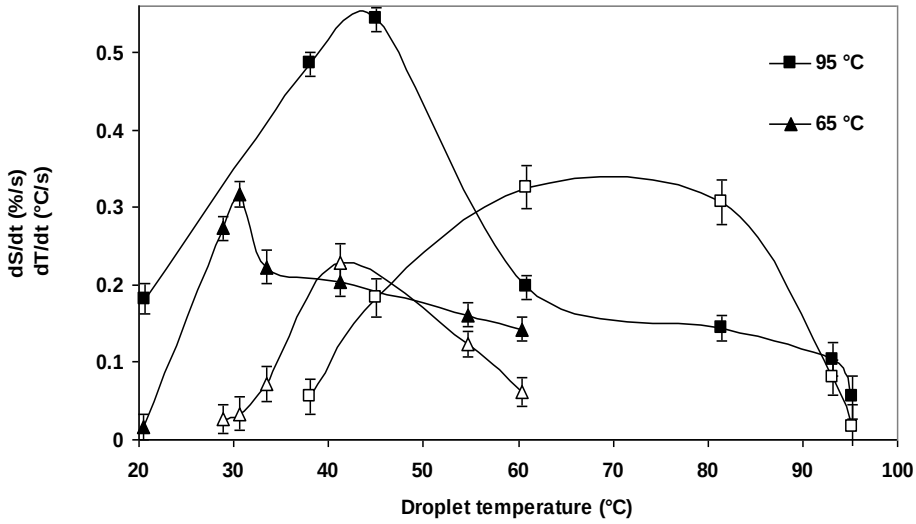


(b)

Fig. 7 Survival of *L. lactis* during single droplet drying as a function of moisture content at different air temperatures (a). The survival of the bacterial cultures in the presence of protectants at the set air temperature of 65°C (b).

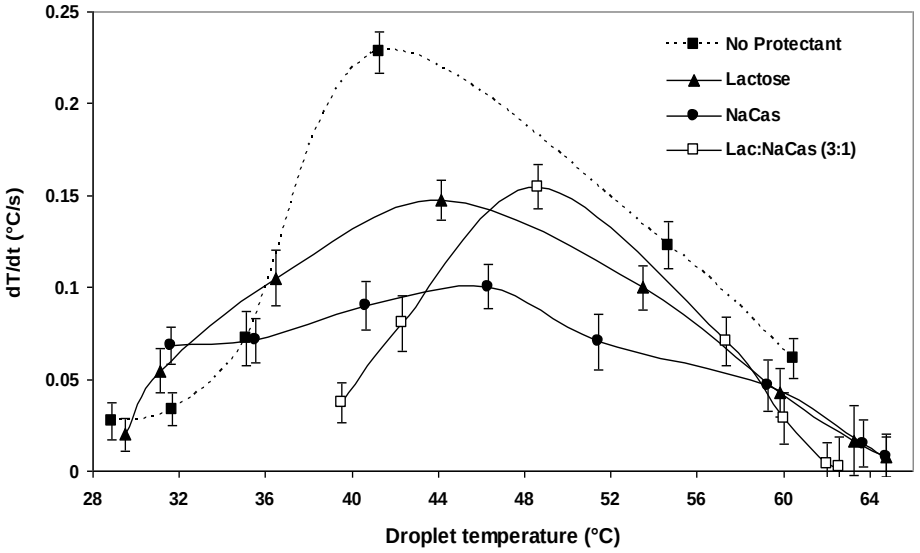


(a)

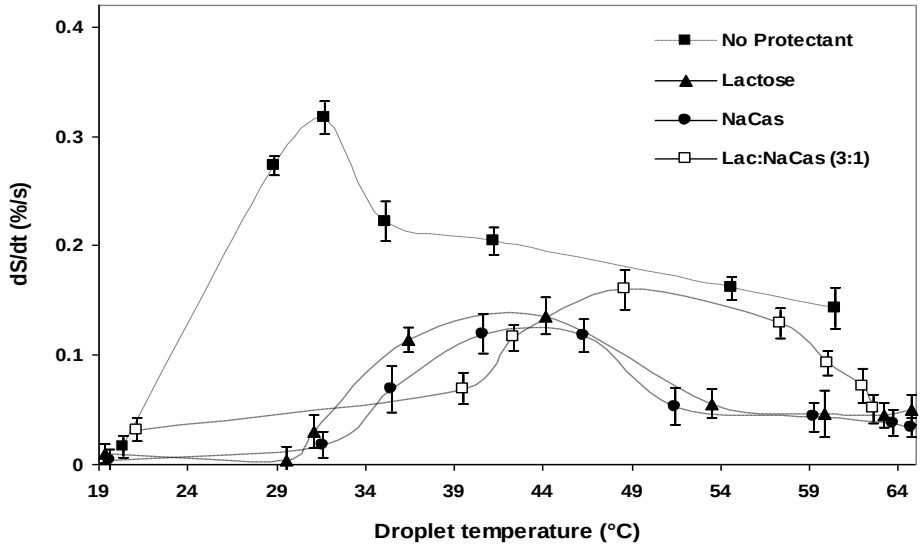


(b)

Fig. 8 The rates of change of survival and droplet temperature of *L. lactis* as a function of droplet temperature during single droplet drying in the absence of protectants at 45°C (a) and 65°C, 95°C (b). (dS/dt (■,▲) and dT/dt (□,Δ)).



(a)



(b)

Fig. 9 The rates of change in droplet temperature (a) and survival (b) of *L. lactis* as a function of droplet temperature during single droplet drying at air temperature of 65°C.