

Effect of lactic acid and galactose on the storage stability of lactose rich  
permeate powders

by

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## Abstract

Deproteinized whey (DPW) and milk permeate (MP) are coproducts obtained during the ultrafiltration of whey and milk, respectively. Then, they undergo unit operations like evaporation, crystallization, and spray drying resulting in a non-hygroscopic, free-flowing powder. Typically, DPW powders contain 76-85% lactose, 2-7% protein, 3-4.5% moisture, and 8-11% ash. On the other hand, MP powders typically contain 78-88% lactose, 3-5% protein, 3-4.5% moisture, and 8-11% ash. These high lactose dairy ingredients are widely used in baked goods, soups, sauces, confectionery, drink mixes, snack foods, beverages, and in certain ice cream formulations. Lactose is present in all dry dairy ingredients, with levels ranging from <2% (caseinates, milk protein isolates) to 100% in lactose powders. Lactose rich powders may undergo chemical and physical changes such as Maillard browning and caking during storage. The presence of lactose in coproducts can adversely affect the powder properties and can lead to stickiness and caking. Furthermore, being a reducing carbohydrate, lactose can also participate in the Maillard reaction causing discoloration of powders during storage. Moreover, the presence of impurities like proteins, peptides, free amino acids, minerals, glucose, and galactose can also significantly influence the shelf-life of lactose rich coproducts. Understanding the effects of various intrinsic and extrinsic factors will improve the stability of lactose-rich-coproducts. This study aimed to investigate the effect of different levels of lactic acid and galactose on the physical, chemical, and thermal properties of DPW and MP powders. For the DPW powder study, two lots of concentrated permeate with total solids of ~77% were procured from a commercial manufacturer and were adjusted to 45% total solids with water and samples were divided into 9 subsamples with varying levels of lactic acid and galactose. For the MP powder study, two lots of MP powders were procured from a commercial manufacturer and were

reconstituted to 45% total solids and this was divided into 4 subsamples with varying levels of lactic acid and galactose. The samples were then dried in a lab-scale spray dryer with inlet and outlet temperatures of 180 and 85°C, respectively. The spray dried DPW powders were sealed and stored at 25°C for 0 (control), 30, 60, 90, 150, 210, and 270 days. The spray dried MP powders were sealed and stored at 25°C (for 0 (control), 60, and 120 days) and 40°C (for 0 (control), 30, 60, 90, 120, and 150 days). At each data point, the powders were analyzed for water activity ( $a_w$ ), color, browning index (BI), FAST index (FI), hygroscopicity, caking, differential scanning calorimetry (DSC), and microstructure. In the DPW powders on storage, the main effect galactose was found to be significantly ( $p < 0.05$ ) different for  $a_w$ ,  $b^*$ , BI, FI, and hygroscopicity and the interaction term lactic acid x galactose was found to be significantly ( $p < 0.05$ ) different for  $L^*$ , FI, and hygroscopicity. On storage, the time and the interaction effects time x galactose, time x lactic acid x galactose each were also found to have a significant ( $p < 0.05$ ) effect on  $a_w$ ,  $L^*$ , and FI values. In MP powders, the main effect lactic acid was significant for  $a_w$ ,  $a^*$ , FI and hygroscopicity at 25°C whereas at 40°C the main effect lactic acid was significant for  $a_w$ ,  $a^*$ ,  $b^*$ , BI and FI. Overall, DPW and MP powders tended to absorb moisture on storage. However, the powders with higher amounts of lactic acid and galactose were more sensitive to absorbing moisture, giving rise to lumping and caking problems. These findings emphasize the importance of controlling the feed characteristics, production process, and storage conditions to reduce the Maillard reaction and limit caking in the final product and to enhance storage stability.

Keywords: Deproteinized whey powder, milk permeate powder, lactic acid, galactose, storage temperature/time

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## Chapter 1 - Introduction

Increasing demand and production of cheese and high-protein dairy ingredients has resulted in the higher production of dairy coproducts including whey permeate, milk permeate, lactose, delactosed permeate, and minerals. Many uses for the coproducts have been identified including applications in animal feeds providing many important nutrients to animals and widely used by dairy ingredient manufacturers in the food and beverage industry. Deproteinized whey (DPW) powder is a coproduct obtained from the ultrafiltration of whey which is widely used in the baking industry due to the presence of high amounts of lactose. It generally consists of 76-85% lactose, 2-7% protein, 3-4.5% moisture, 0-0.1% fat, and 8-11% ash. Many uses have been found for DPW powder such as in bakery products, dry mixes, snack foods, and confections. Some of the compositional differences in whey permeate, including the presence of lactic acid, other sugars (galactose), and minerals, impact moisture absorption behavior by lowering the glass transition temperature. Indeed, lower glass transition temperature makes them hygroscopic and hinders efficient spray drying, and the dried powder is more susceptible to caking during storage. Lactic acid content produced from live cheese cultures can vary due to cheesemaking practices, resulting in variations in the whey permeate powders. Whey permeate has a similar composition to milk permeate. However, milk permeate is derived directly from milk and has fewer processing steps. Milk permeate powders have a minimum of 76% lactose, a maximum of 14% ash and typically between 2-7% protein and are known for its clean/consistent flavor. The presence of lactose in these powders can adversely affect powder properties which can cause undesirable changes such as, stickiness, and caking during processing/storage. Moreover, lactose being a reducing sugar could take part in Maillard reactions which could cause browning in dairy powders. Therefore, to minimize processing and product quality losses, coproducts with large

amounts of lactose, such as milk and whey permeates, are subjected to a crystallization step before drying to convert most of the amorphous lactose into the more stable, crystalline form. However, it is not possible to fully crystallize the dissolved lactose and the resulting powders may contain both lactose crystals and a proportion of amorphous lactose in addition to other hygroscopic milk components (Bansal and Bhandari, 2016). Hygroscopicity is closely linked with stickiness, and as the moisture content increases the rate of stickiness development increases (Murti et al., 2009). Stickiness and hygroscopicity of high lactose powders are major challenges for the dairy industry, particularly during the spray drying process and during storage (Huppertz and Gazi, 2016). Stickiness leads to wall depositions, decreasing process efficiency, product yield, and quality. Presently, there is no published literature available on the detailed effects of lactic acid and galactose on the storage stability of these powders. With the increased interest in using whey and milk permeate powders in the food and animal industry, knowledge of the key factors affecting their multi-component system and insights on its storage stability could be helpful in optimizing their processing, handling, and storage.

## **References**

- Bansal, N., and Bhandari, B. 2016. Functional milk proteins: Production and utilization—whey-based ingredients. In *Advanced dairy chemistry*. Springer, New York, NY. 67-98.
- Huppertz, T., and Gazi, I. 2016. Lactose in dairy ingredients: Effect on processing and storage stability. *J. Dairy Sci.* 99: 6842-6851.
- Murti, R. A., Paterson, A. T. H., Pearce, D. L., and Bronlund, J. E. 2009. Stickiness of skim milk powder using the particle gun technique. *Int. Dairy J.* 19: 137-141.

## Chapter 2 - Literature Review

### Overview of lactose rich coproducts

The US production of high protein milk and whey ingredients continues to grow, as the global demand increases. The growing concern over environmental pollution has escalated the research in cheese whey utilization (Hargrove et al., 1976). Also, the increased production of high-protein dairy ingredients due to the increased demand has contributed to the higher production of coproducts including whey permeate, milk permeate, lactose, delactosed permeate (DLP), whey phospholipid concentrate (WPLC) and milk minerals. Some of the lactose rich products include milk permeate powders (82% lactose), demineralized whey powders (DMW; 78% lactose), reduced lactose whey powders (RLW; 51% lactose), skim milk powders (SMP; 51% lactose). Table 2.1 shows the proximate composition of some of the lactose rich coproducts. The processing flow chart of milk permeate powders and whey permeate powders are shown in Figure 2.1.

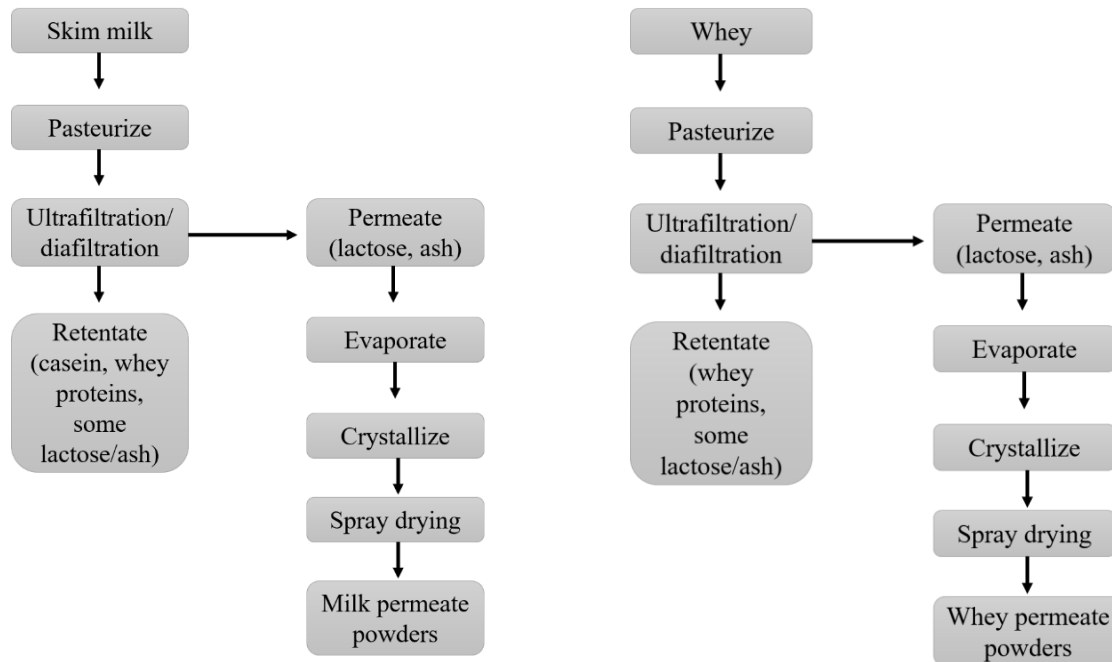
**Table 2.1** Proximate composition of lactose rich coproducts

Lactose rich coproducts	Composition (wt. %)				
	Lactose	Protein	Ash	Fat	Moisture
Milk permeate powder	82	3	9	<1	4
Deproteinized whey powder	80	4	9	0.2	4
90% Demineralized whey powder	80	13	1	1	5
50% Demineralized whey powder	78	13	3	1	5
25% Demineralized whey powder	77	12	5	1	5
Reduced lactose whey powder	56	23	16	2	3
Mineral concentrated whey powder	56	22	17	2	3
Skim milk powder	51	36	8.2	0.8	4

Composition adapted from Smith (2017)



Lactose is the principal carbohydrate present in milk (Refer Figure 2.1), and it is present in all dairy ingredients from concentrations ranging from less than 2% to very high concentrations as in pure lactose powders. Lactose is a disaccharide comprising of D-glucose and D-galactose which are linked together by  $\beta$ -1,4-glycosidic linkage. It is a reducing sugar due to the presence of a free aldehyde group. Lactose exhibits polymorphism i.e. it has the ability to occur in several different forms. Lactose can exist in four forms:  $\alpha$ -lactose-monohydrate, stable  $\alpha$ -lactose anhydrous, unstable  $\alpha$ -lactose anhydrous, and  $\beta$ -lactose anhydrous. Also, the  $\alpha$ - and  $\beta$ -lactose anomers have the ability to change from one form to another, referred to as mutarotation. The complexity and the different chemical and physical properties exhibited by lactose could be explained by polymorphism and mutarotation (Hourigan et al., 2013).



**Figure 2.1** Processing flow chart of milk permeate powders and whey permeate powders.

The presence of lactose in dairy powders can adversely affect powder properties which can cause some undesirable changes such as stickiness and caking during storage. Caking can happen at any point during manufacture, transportation or during storage and can be attributed to different factors. Also, lactose being a reducing sugar could take part in Maillard reactions which

could cause browning in dairy powders. Various factors attribute to this such as the state of lactose, storage temperature, storage time, relative humidity (RH), etc. Therefore, it is very critical to understand the processing conditions and storage changes happening to the powders which help in the handling, processing, and storage of the lactose rich powders.

This review deals with the effect of different processing conditions and different storage parameters, such as temperature, time, RH that cause the caking and browning of lactose rich products.

### **Lactose and its forms**

Lactose is the main carbohydrate present in the milk of most mammals. Lactose is a disaccharide and can occur in both  $\alpha$  and  $\beta$  forms. These are stereoisomers that differ only by their spatial arrangement of the hydroxyl group on the first carbon of the glucose moiety. Lactose is a reducing sugar due to the presence of the aldehydic functional group. The two stereoisomers  $\alpha$ - and  $\beta$ - lactose anomers in solution has the ability to interconvert from one form to the other by a spontaneous process called mutarotation (Wong and Hartel, 2014). The complex chemistry of the lactose molecule can be attributed to polymorphism (ability to crystallize into different forms) and mutarotation. The four polymorphs of lactose are  $\alpha$ -lactose monohydrate, stable and unstable forms of  $\alpha$ -lactose anhydrous, and  $\beta$ -lactose anhydrous. Also, lactose can be found both in an amorphous and crystalline state or as a mixture of both. The crystalline form of lactose has a very ordered structure whereas, the amorphous form is not ordered to a regular lattice (Hourigan et al., 2013).

The most stable and common form of lactose is the  $\alpha$ -lactose monohydrate due to the presence of one molecule of water of crystallization. The water molecule is critical as it links together oxygens of four lactose molecules and plays a major role in the structure and

stabilization of  $\alpha$ -lactose monohydrate (Carpin et al., 2016). When  $\alpha$ -lactose monohydrate eliminates water of crystallization from its crystal structure, the stable and unstable forms of anhydrous lactose are formed. The arrangement of the crystal structure is left unchanged for the unstable form of  $\alpha$ -lactose anhydrous whereas, the stable form of  $\alpha$ -lactose anhydrous has a different structure. Both the forms can be produced when  $\alpha$ -lactose monohydrate crystals are heated at higher temperatures between 100 and 190°C, preferably under vacuum. The unstable form of  $\alpha$ -lactose anhydrous is formed at lower temperatures (100-130°C) whereas the stable form of  $\alpha$ -lactose anhydrous is formed at higher temperatures (160-170°C) as rearrangement occurs due to the availability of thermal energy (Hourigan et al., 2013). When both the  $\alpha$  and  $\beta$  anomers exist in the same crystal lattice in different molar ratios, it is characterized as the compound crystals of anhydrous  $\alpha$ - and  $\beta$ - lactose. The currently known forms of lactose is as shown in Table 2.2.

**Table 2.2.** Currently known forms of lactose

	Monohydrate	$\alpha$ -lactose
		$\alpha$ -lactose unstable
Crystalline	Anhydrous	$\alpha$ -lactose stable
		$\beta$ -lactose
		Compound $\beta/\alpha$ lactose
Amorphous		Mixture of $\alpha$ - and $\beta$ -lactose

Adapted from Listiohadi et al. (2005)

The only  $\beta$  anomeric form of crystalline lactose known to exist is anhydrous  $\beta$ -lactose. It can be crystallized either from  $\alpha$ -lactose monohydrate or from a saturated lactose solution by different ways in which most of the methods involve heating the saturated solution at high temperature. It has been reported that the ideal or threshold temperature above which  $\beta$ -lactose can be crystallized out from aqueous systems is 93.5°C (Hudson, 1904; Carpin et al., 2016).

Commercially, roller drying of saturated lactose solution above 93.5°C produces anhydrous lactose high in  $\beta$  (Lerk, 1993; Hourigan et al., 2013). However, Vu et al. (2009) have reported that the production of  $\beta$ -lactose is possible at a temperature lower than 93.5°C.

Amorphous lactose is the most hygroscopic of all the forms of lactose and does not have an ordered arrangement in a regular lattice. Amorphous lactose occurs as a concentrated low moisture syrup in the glassy state and is thermodynamically unstable. It is usually obtained during the spray drying of lactose solution when a rapid increase in viscosity occurs and there isn't enough time for the crystallization to happen (Holsinger, 1997). It has been reported that some of the processes like milling and grinding generally produces amorphous lactose in the range of 0.5 to 0.6% of total lactose (Listiohadi et al., 2005).

## **Properties of lactose in solution**

### **Solubility**

Lactose has a poor solubility compared to other sugars (Harju, 2001). The rate of dissolution of lactose depends on the type of lactose used as the  $\alpha$  and  $\beta$  forms have different solubilities. The solubility of  $\alpha$ -lactose in water is lower when compared to the  $\beta$ -lactose. When a surplus amount of  $\alpha$ -lactose monohydrate is added to water about 7g/100g dissolves at 15°C which is the true solubility of this form of lactose. However, with time the solubility increases due to mutarotation. This happens when  $\alpha$  form gets converted to  $\beta$  allowing more of  $\alpha$ -lactose to dissolve as the solution becomes unsaturated of  $\alpha$ -lactose. This process persists until a final equilibrium is reached and thus the final solubility of  $\alpha$ -lactose becomes about 17g/100g at 15°C. Under similar conditions, the solubility of  $\beta$ -lactose exhibits higher initial solubility of 50g/100g at 15°C (Holsinger, 1997). It was reported that the solubility of  $\alpha$ -lactose increases with temperature (Walstra et al., 2005; Paterson, 2009; Huppertz and Gazi, 2016). At any

temperature, the supersolubility of lactose is equivalent to the saturation value at a temperature higher than 30°C (Holsinger, 1997). Lactose solubility can be affected by various other constituents of milk. It has been noted that the lactose solubility in pure solutions is higher than those of dairy liquids. It has been found that the addition of lactic acid or lactate and sodium phosphate reduces the lactose solubility which could be explained by the water-structure-making capability of phosphates (Smart and Smith, 1992; Huppertz and Gazi, 2016). However, the addition of potassium phosphate increased the solubility as the water-structure-making capability of phosphates was overpowered by the water-structure-breaking capability of potassium. Thus, the solubility of lactose can be low in whey due to the presence of impurities (Bhargava and Jelen, 1996; Wong and Hartel, 2014).

### **Mutarotation**

Mutarotation, as the term suggests, is the change in optical rotation. It is a very important property to take into consideration while studying about lactose. The conversion of the two anomeric forms of lactose (i.e.  $\alpha$ -lactose and  $\beta$ -lactose) occurs through the open-chain form. Lactose undergoes mutarotation as it contains a free anomeric carbon, enabling it to equilibrate into the anomeric forms. It was reported that the mutarotation of lactose follows a first-order reaction (Haase and Nickerson, 1966). When mutarotation occurs one form to the other, the optical rotation will change until a specific rotation of +55.3°. In water at 20°C, the specific rotation of  $\alpha$  and  $\beta$  forms is + 89.4° and + 35°, respectively. At equilibrium  $[\alpha]^{20}_D = + 55.3^\circ$ , representing ~37.3%  $\alpha$ - and ~62.8%  $\beta$ -lactose, respectively. Therefore, the equilibrium ratio of  $\beta$  to  $\alpha$  at 20°C is 1.68. The  $\alpha$ -lactose and  $\beta$ -lactose equilibrium in aqueous solutions are dependent on concentration and temperature. However, pH does not affect the mutarotation equilibrium but affects the rate at which the equilibrium is being established. The rate of mutarotation increases

with the increase in temperature. Walstra and Jenness, (1984) reported that for every 10°C rises in temperature there is an approximately 2.8-fold increase in the rate of mutarotation. Also, the mutarotation rate was found to be affected by the presence of milk salts (Huppertz and Gazi, 2016).

## **Supersaturation**

The supersaturation of lactose can be defined as the state in which the solution contains more of dissolved lactose molecules than that is thermodynamically permitted. Lactose solutions can turn out to be profoundly supersaturated before crystallization happens. Supersaturation is the driving force for crystallization to happen. There are four regions of supersaturation that exists i.e. the labile zone, the intermediate zone, the metastable zone, and the undersaturated zone. The region where spontaneous nucleation occurs at high supersaturation levels is the labile zone. Underneath this region is the narrow intermediate zone, nucleation will happen only when seed crystals are available known as the secondary nucleation. The metastable zone displays the region where seed crystals will grow but nucleation will not happen at lower supersaturations. The undersaturated zone represents the region where neither nucleation nor crystal growth can occur (Wong et al., 2011; Hourigan et al., 2013).

## **Lactose crystallization**

Different types of lactose crystals can be formed, but under conventional industrial conditions, the principle crystalline form in which lactose is found is the  $\alpha$ -lactose monohydrate form. This is the most stable form of lactose due to the water molecule attached and accounts for 5% of the formula weight. The  $\alpha$ -lactose monohydrate crystallizes out at temperatures below 93.5°C whereas above this the anhydrous  $\beta$ -lactose crystals are obtained (Hourigan et al., 2013). However, when water removal during spray drying is very rapid that there no time for the

crystallization to happen, the amorphous lactose is formed. It is the most hygroscopic form due to which it absorbs moisture to the point that crystallization to  $\alpha$ -lactose monohydrate can happen owing to the increased molecular mobility (Huppertz and Gazi, 2016).

Crystallization is a process that involves steps namely nucleation and crystal growth. Supersaturation acts as the driving force for crystallization to happen. Nucleation can be classified as primary and secondary nucleation. Primary nucleation can be defined as the formation of the first nucleus whereas secondary nucleation refers to the crystal nuclei being formed from an already existing crystal (Wong and Hartel, 2014). Secondary nucleation could be initiated at even low supersaturation levels as it has lower activation energy (McLeod, 2007). Induction time is frequently used to describe the rate of nucleation. Induction time refers to the time elapsed between the inception of supersaturation to the development of nuclei. The crystal growth is a three-step process that includes mass transport, surface reaction and transport of latent energy away from growing crystals (Wong and Hartel, 2014). Crystal growth is affected by various factors such as supersaturation, temperature, pH, viscosity, and the presence of impurities (Bhargava and Jelen, 1996). The most important parameter affecting crystal growth is supersaturation whereas others have an indirect effect. The presence of proteins or other higher molecular weight compounds can delay the lactose crystallization process (Haque and Roos, 2004; Jouppila and Roos, 1994; Thomas et al., 2004). The presence of organic acids such as acetic and lactic acid has an inhibitory effect on lactose crystallization (Jelen and Coulter, 1973; Chandrapala et al., 2016). It is ideal to crystallize lactose from sweet whey as it has a higher pH when compared to acid whey (Modler and Lefkovitch, 1986; Wong and Hartel, 2014). Mimouni et al., (2005) reported that the presence of whey protein significantly lowered the final lactose

crystal size but promotes nucleation. Various other inhibitors of lactose crystallization include riboflavin,  $\beta$ -lactose and lactose monophosphate (Walstra and Jenness, 1984).

## **Properties of lactose in dairy powders**

### **Water sorption**

Powder handling issues related to lactose are frequently as a result of water sorption by lactose. The key step is to distinguish between crystalline and amorphous lactose while taking into account the moisture sorption of lactose (Huppertz and Gazi, 2016). Crystalline lactose is reported to have a significant amount of water sorption at water activities over 0.85 and shows very little water sorption at a water activity ( $a_w$ ) below 0.85. Additionally, the moisture content increase is exponential above this point due to capillary condensation (Bronlund and Paterson, 2004). It has been investigated that in the temperature range of 12-40°C there is no significant effect of temperature on the sorption isotherm for crystalline lactose. In the case of amorphous lactose, it has been shown that there is a negligible influence of temperature in these regions however is significantly higher than that of crystalline lactose (Jouppila and Roos, 1994b; Bronlund and Paterson, 2004; Huppertz and Gazi, 2016). There is a significant decrease in the moisture sorption isotherm if a small quantity of amorphous lactose is present on a crystalline lactose powder. When rapid drying of the surface moisture on sugar crystals or when milling operations are performed amorphous surfaces on crystalline sugars can be formed (Roth, 1976). The moisture uptake follows a linear trend for amorphous lactose with increasing  $a_w$  up to 0.3. Due to amorphous lactose crystallization that happens above the glass transition temperature, the moisture uptake decreased at water activities above 0.4 (Roos and Karel, 1990).

Moisture plays a crucial part in the glass transition and crystallization behavior of amorphous powders that decide its flowability, stickiness, and storage stability. In a study



conducted by Shrestha et al. (2007), it was reported that the skim milk powder hydrolyzed with lactose absorbed less water when compared to lactose at each range of  $a_w$  up to 0.43. Also, lactose began to lose sorbed water at  $a_w$  over 0.43 due to the crystallization of lactose but for the skim milk powder hydrolyzed with lactose, crystallization did not happen even at  $a_w$  as high as 0.75 which shows that the water sorption behavior of skim milk powder was affected by hydrolyzed lactose. Although, the spray-dried skim milk powder when stored for a week started to crystallize at  $a_w \geq 0.43$  (Shrestha et al., 2006). It has been previously reported that there was the loss of sorbed water at various RH conditions in skim milk powder due to lactose crystallization (Bushill et al., 1965; Lai and Schmidt, 1990; Jouppila and Roos, 1994a; Jouppila and Roos, 1994b). Moreover, it was found that amorphous lactose in skim milk powder crystallized as an anhydrous mixture of  $\alpha$ - and  $\beta$ -lactose in a molar ratio of 5:3 for skim milk powder stored at 55% RH. However, other crystal forms were also present at high RH conditions (Jouppila et al., 1997).

It has been reported that different forms of lactose have significantly different moisture sorption characteristics. The amount of water absorbed was primarily dependent on the relative humidity, temperature, and time of storage. However, relative humidity had more effect on moisture sorption when compared to the time. In a study conducted by Listiohadi et al. (2008), the moisture sorption characteristics of powders which contained polymorphs of lactose and amorphous lactose at various levels of RH were investigated. The polymorphic and isomeric properties of lactose play an important role in the hygroscopicity and caking of dairy powders. The moisture uptake of  $\alpha$ -lactose monohydrate happened quickly at all levels of RH during storage. The  $\alpha$ -Lactose anhydrous stable was found to be very hygroscopic at 75% RH but was less hygroscopic below 57% RH. However, for  $\alpha$ -lactose anhydrous unstable the moisture

absorption increased during storage with the increase in RH from 33% to 75%. Holsinger (1988) reported that the  $\alpha$ -lactose anhydrous unstable could easily accommodate absorbed water to convert to  $\alpha$ -lactose monohydrate whereas  $\alpha$ -lactose anhydrous stable could not without dissolving in water both attributing to their respective crystal structures. The rate of crystallization was negatively affected by the presence of milk fat, but the amount of water absorbed after complete crystallization became constant and was independent of fat content. Lactose crystallization in milk powders was monitored by water plasticization and depression of the glass transition temperature ( $T_g$ ) to below ambient temperature (Jouppila and Roos, 1994a).

### **Phase transitions of lactose**

Phase transitions in lactose can be a result of moisture absorption in dairy powders. It has been discussed earlier that when rapid water removal happens during spray drying and there is not enough time for crystallization to happen, then amorphous lactose is being formed. This amorphous lactose appears as a concentrated low moisture syrup in the glassy state. And, the temperature at which the gradual transition happens in amorphous materials from a hard, glassy state to a rubbery state is called the  $T_g$ . Various physicochemical changes that happen in dairy powders are directly or indirectly a result of the glass transition of lactose in the amorphous form. A drive towards the thermodynamic equilibrium will happen as the glassy state is a nonequilibrium state. Also, in the glassy state, the molecular rotations and vibrations are limited as the molecular mobility is restricted (Roos, 2002). Differential scanning calorimetry (DSC) is a thermoanalytical technique that can be used to determine glass transitions which appear as endothermic changes in heat capacity. A DSC measures the difference in heat flow rate between a sample and reference as a function of temperature and time. Generally, glass transitions are depicted by an onset, midpoint, and endpoint temperature (Roos, 2002; Roos, 2010).

Anhydrous lactose has an onset  $T_g$  of 101°C (Roos and Karel, 1991). It was observed that milk powders containing lactose had a  $T_g$  close to that of pure lactose. However, in milk powders with hydrolyzed lactose, the  $T_g$  decreased significantly which could be attributed to the low  $T_g$  of glucose and galactose (Jouppila and Roos, 1994b). Amorphous lactose can be plasticized by water due to which the  $T_g$  decreases (Roos, 2010). The water sorption and  $T_g$  of milk powders with hydrolyzed lactose and skim milk powder with hydrolyzed lactose have been studied. The  $T_g$  of milk powder, skim milk powder and milk powder with hydrolyzed lactose at 11% RH was observed to be 61°C, 62°C and 36°C, respectively (Fernández et al., 2003). And, the  $T_g$  of skim milk powder with hydrolyzed lactose was observed as 49°C (Shrestha et al., 2007). The  $T_g$  value reported by Jouppila and Roos (1994b) for anhydrous hydrolyzed lactose skim milk powder was also 49°C. In a study conducted by Haque and Roos (2004), it was reported that whey protein isolate (WPI), gelatin, and albumin increased the  $T_g$  of dry powders whereas Na-caseinate decreased the  $T_g$ .

Generally, dairy powders exist in the amorphous glassy state at very low water content. However, as the water content increases and water being a plasticizer converts the amorphous structure from a glassy state to a rubbery state which results in a decrease in  $T_g$  (Roos and Karel, 1990). Lactose crystallization, stickiness, caking and collapse in dairy powders have been characterized in the rubbery state (Chuy and Labuza, 1994). Lactose crystallization can occur at around 0.3 to 0.4 RH when enough moisture is absorbed, and the  $T_g$  is reduced below ambient. This could be characterized by a weight loss as crystalline forms of lactose has lower amounts of water than amorphous lactose (Huppertz and Gazi, 2016). It was reported that lactose crystallization occurred slower in skim milk powder when compared to pure lactose which could be due to the presence of milk proteins and salts delaying lactose crystallization in skim milk

powder Jouppila and Roos (1994a,b). In partially crystalline whey powders, crystallization of amorphous lactose was delayed due to the presence of protein and salts in the non-crystalline powder matrix (Ibach and Kind, 2007; Nijdam et al., 2008).

### **Problems associated with the presence of lactose in dairy powders**

Lactose is the principal constituent of dairy powders like skim milk powder, deproteinized whey powder, milk permeate powder and is also available as pure crystalline lactose. Nowadays, lactose rich coproducts are used in a wide range of applications such as the formulation of food products like bakery products, infant formulas, energy drinks, and is also widely used in the pharmaceutical industry. Thus, the production of dairy powders with lactose as the main ingredient, including lactose powder has increased globally. Increased production of lactose and lactose coproducts raises the concern of effective processing (state of lactose, drying parameters) and storage conditions (storage temperature, RH) to maintain a good quality powder. Effective storage conditions are determined based on the factors like galactose, proteins, lactic acid, and minerals; dairy powder properties like particle size; spray drying parameters like inlet temperature. Lactose rich coproducts such as deproteinized whey powders, milk permeate powders, etc., may undergo some undesirable changes such as caking and Maillard browning during storage due to its high lactose content. The biochemical and physicochemical changes like Maillard reaction, lactose crystallization, and caking that occur during storage alter the physical and functional properties of the powder. Lactose being a reducing sugar can take part in the Maillard reaction which causes browning in the powders. Thus, it is very important to know in detail about the stickiness, caking and clumping mechanisms and about the Maillard browning that can happen in lactose rich powders.

## **Influence of lactose on dairy ingredient manufacture and powder properties**

Stickiness and caking are deleterious to dairy powders which cause problems during processing, storage, and handling and are affected by lactose. Stickiness can be defined as the sticking of powder particles to the dryer or fluid bed and can be observed during drying. Caking in simple terms can be defined as the formation of lumps in a free-flowing powder. Stickiness happens before caking and is associated with the formation of solid bridges formed between particles (Listiohadi et al., 2005; Kelly, 2009). Environmental factors such as temperature, RH can accelerate these processes. There are several other factors such as the glass transition of lactose that is related to the stickiness and caking of dairy powders (Listiohadi et al., 2005; Kelly, 2009; O'Callaghan and Hogan, 2013).

### **Lactose induced stickiness**

Stickiness could be described as a surface property in which liquid bridges are formed as a result of surface energy-driven viscous flow during particle contact (Downton et al., 1982). The thermodynamically unstable amorphous lactose possesses a driving force to crystallize, provided that the molecules can move. When dairy powders are exposed to higher temperature and humidity conditions, they can mobilize as a high viscosity flow which leads to stickiness and further lead to caking (Aguilera et al., 1995). Stickiness occurs if there are adequate liquid bridges and the formed liquid bridge is strong enough to withstand deformation. Viscous flow does not occur in the glassy state however, as the temperature increases the viscosity decreases above the  $T_g$  (Roos, 2010; Paterson et al., 2015).

Stickiness can be a significant issue during spray drying. When the particles are not dried adequately during drying, particles can collide with each other or with the chamber walls of the drying apparatus thus become sticky. This, in turn, can lead to low product yields, operating



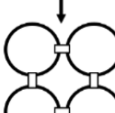
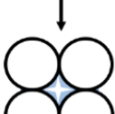

problems, and issues during powder handling. These problems are more prevalent in materials containing a high amount of hygroscopic, amorphous sugars. It has been reported that flushing with cool air and cooling the dryer walls have been used to avoid stickiness in spray dryers (Lazar et al., 1956). Various high molecular weight polymers have also been used to combat stickiness in spray drying (Brennan et al., 1971).

### **Lactose induced caking**

Stickiness, caking, clumping, and ultimately collapse is critical in terms of the physical stability of the dairy powder, which result in free-flowing non-hygroscopic powder to form lumps, then into an agglomerated solid, which finally turns into a sticky material on exposure to higher humidity and temperatures (Adhikari et al., 2001). Caking is a broad term that is often difficult to define and depends on factors such as moisture, temperature and comprise of different stages such as bridging, agglomeration, compaction, and liquefaction. Depending on different stages various factors such as the number of lumps formed, lump size, and the degree of hardness can vary. The stages involved in a caking process is shown in Figure 2.2. Caking index, a term coined by Aguilera et al., (1995) can be defined as the state of a system at any time with respect to an initial state. The ratio of interparticle bridge diameter to particle diameter,  $D_{bridge}/D_{particle}$ ; and the ratio of instant system porosity to initial system porosity,  $p(t)/p_o$  can be described as the morphological indicators of the state of the system.

Surface deformation and sticking in contact points between particles lead to bridging, the initial stage in caking. The next stage is agglomeration, in which particle clumps with structural integrity and involves an irreversible consolidation of bridges. Compaction is a more advanced stage of caking where there is a notable loss of system integrity. This can be related to the thickening of interparticle bridges, reduction in interparticle spaces and deformation of particle

clumps under pressure. Liquefaction, which is the final stage of caking, these interparticle bridges formed disappear as a result of liquefaction of sample and extensive flow owing to high moisture content (Aguilera et al., 1995).

Stage	$p(t)/p_o$	$D_{bridge}/D_{particle}$	Morphology
Free flowing	1	0	
Bridging	1	0	
Agglomeration	<1	>0	
Compaction	0	1	
Liquefaction	0	1	

**Figure 2.2** Stages during the typical caking process, indicating the changes in system porosity  $p(t)/p_o$  and the ratio of interparticle bridge diameter to particle diameter at each stage  $D_{bridge}/D_{particle}$  (Adapted from Aguilera et al., 1995).

When dairy powders contain lactose in its amorphous state, it can crystallize producing a solid discontinuous lactose phase within particles. For most powders,  $T_g$  is well above the storage temperature. When powder temperature is greater than the  $T_g$ , then only crystallization will take place as the molecules have enough mobility to initiate crystallization (Jouppila and Roos, 1994b; Jouppila et al., 1997; Roos 2003). Amorphous lactose present in the powder absorbs moisture which causes the water to increase which results in plasticization and decrease in  $T_g$ . When  $T_g$  is reduced below the powder temperature crystallization will occur. However,

there can be the formation of liquid bridges before crystallization which leads to an increased tendency of cohesiveness and caking of the powder. When powders are exposed to higher temperatures and relative humidities, leading to a reduction of  $T_g$  thus causing powder handling problems (Fitzpatrick et al., 2007).

### **Different mechanisms of caking**

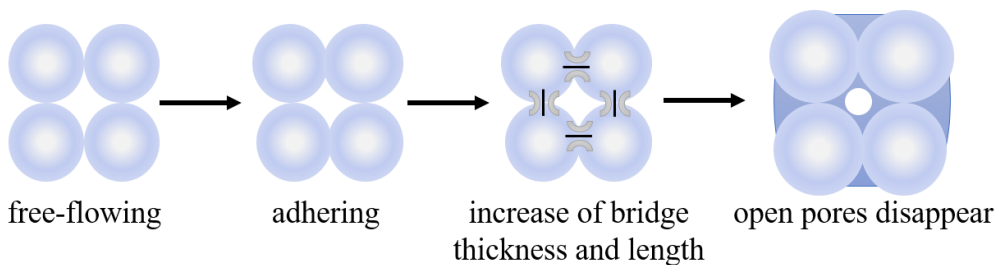
Caking is a recurrent problem in the dairy industry for many years. Caking can be defined in simple terms as the formation of lumps in powdered material. Caking can occur when particles in the powder agglomerate either due to the presence of adhesive material among particles or due to particle-particle electrostatic attraction. Various factors contribute to the caking of dairy powders which include temperature, RH, presence of amorphous material, particle size and, the cohesiveness of powder (Peleg, 1977). Other factors that influence caking are compression or high pressures (Adhikari et al., 2001). The general mechanism of caking in dried milk involves three steps: (1) absorption of moisture by lactose (2) agglomeration of the particles (3) crystallization and solidification of some of the lactose (Troy and Sharp, 1930). There are three mechanisms in which caking takes place. The first one involves crystallization of amorphous material whereas the second one is due to the deliquescence of crystalline particles and moisture plays an important part in both. The third mechanism is due to the balance of forces between particles and does not involve moisture (Carpin et al., 2016).

### **Amorphous caking**

When dairy powders containing amorphous lactose are stored in a high RH environment, it tends to absorb water which in turn decreases the viscosity (Hartmann and Palzer, 2011). Sintering is the main adhesion mechanism in which powders with amorphous lactose cakes and can be thus referred to as amorphous caking. Sintering can be defined as the ability to make a



powder material coalesce (come together and form one mass) into a porous or solid mass either by heating or compressing. A schematic diagram of the sintering process is shown in Figure 2.3. However, this process does not involve liquefaction. As the water content increases, water-soluble amorphous lactose does not dissolve like crystalline lactose, but water migrates into the amorphous molecular matrix (Hartmann and Palzer, 2011). Water being a plasticizer causes the amorphous lactose to change from the glassy state to the rubbery state and thus reduces the  $T_g$ . Thus, a decrease in viscosity occurs which allows the flow of material to contact points. Solid bridges are formed between particles due to the crystallization of amorphous lactose if the temperature is increased further.



**Figure 2.3** Schematic diagram of sintering process (Adapted from Hartmann and Palzer, 2011).

Amorphous lactose present in dairy powders being very hygroscopic absorbs water when stored in a high humidity environment. The absorbed water, in turn, penetrated the liquid into the capillaries and diffusion of moisture occurs into the amorphous matrix (Hartmann and Palzer, 2011). The water molecules are stored in the free volume left in this matrix. Thus, with the increase in capillaries filled with water, there exists an increased surface area for moisture migration into the solid matter. This humidified solid undergoes glass transition and the amorphous lactose migrates into the water on the particle surface. This results in a significant increase in the viscosity of moisture remaining on the particle surface. This increase in viscosity due to the dissolution of a solid substance reduces the velocity of liquid penetration which in turn generates a viscous solution on the particle surface. When such a particle comes in contact with

another particle a viscous bridge is formed between the particles due to the viscous flow (Palzer, 2005). Additionally, when the mutual contact point of the amorphous lactose particles are near or above the glass-rubber transition point, viscous flow occurs (Renzetti et al., 2012). This viscous flow caused due to surface tension or pressure which leads to the formation of a bridge is called sintering. Sintering can also happen when powder particles with enough moisture are stored for a long period (Palzer, 2005).

An equation was developed by Frenkel (1945) taking into account the sintering process which gives the calculation of the ratio between the diameter of the sinter bridge  $X$  and the diameter of particle  $a$ . This equation involves the influence of applied force  $F_t$  on the sintering, velocity can be given by:

$$\left(\frac{X}{a}\right)^2 = \left(\frac{4\gamma}{5a} + \frac{2F_t}{5\pi a^2}\right) \frac{t}{\eta} \quad (1)$$

Where  $\gamma$  surface tension

$F_t$  applied force

$t$  time

$a$  diameter of the particle

$X$  diameter of the sinter bridge

$\eta$  dynamic viscosity

$\eta^g$  dynamic viscosity at the glass transition temperature

Various factors such as the size of the contact area, contact time and force between particles play a major role during sintering (Renzetti et al., 2012). Many authors have suggested models describing agglomeration as a function of the aforementioned variables. The contact time between amorphous particles needed to create sufficient adhesion force due to sintering which can lead to caking can be calculated by the given equation (Palzer, 2005):

$$t = \left( \frac{5a^2\pi}{4\gamma a\pi + 2F_t} \right) \eta^g \left( \frac{X}{a} \right)^2 10^{[C(T-T_g)]/[B+(T-T_g)]}$$

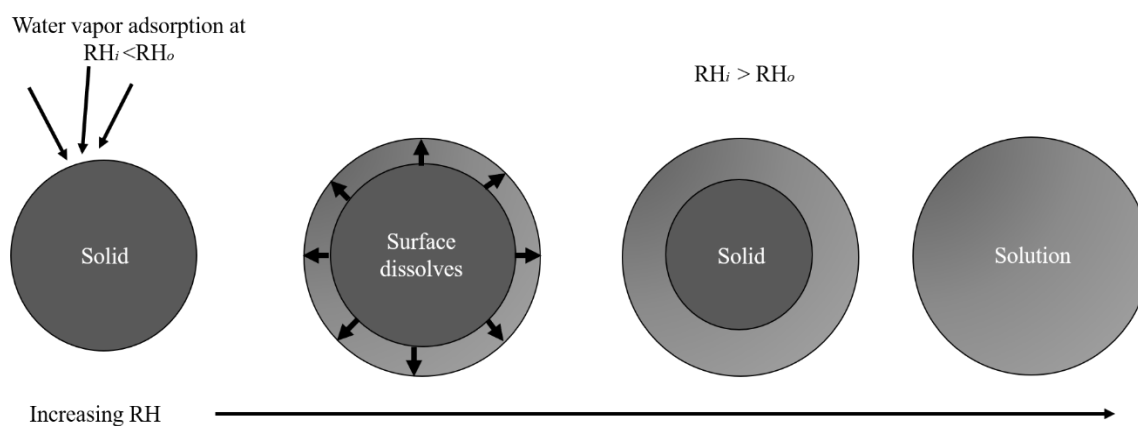
with  $\left(\frac{X}{a}\right)^2 = 0.1$  and  $\eta^g = 10^{12} Pa s$ . (2)

Another important factor that affects the stability of amorphous material is  $T_g$ . The rate of crystallization and the degree of caking is influenced by the difference between the storage temperature and onset  $T_g$  of the material ( $T - T_g$ ) (Foster et al., 2006; Roos and Karel, 1993). Thus, knowing the  $T_g$  of the powder, the time at which stickiness or caking occurs can be calculated by the above-mentioned Eq. (2). Descamps and Palzer (2007) illustrated that there was a sinter bridge formation between two maltodextrin particles at 76% RH at 25°C for 2 hours with the help of light microscopy. Sinter bridge formation is the main caking mechanism for maltodextrin. When  $T_g$  was reduced below the storage temperature, the sintering is observed where molecules have enough mobility to flow in between the gaps of the neighboring particles and form a sinter bridge (Descamps and Palzer, 2007).

### **Humidity caking**

Food/ dairy powders need to be free-flowing to ease powder transportation, mixing, and formulations. A free-flowing powder is converted to a lumpy aggregate when it cakes (Aguilera et al. 1995). The caking caused by the formation of liquid bridges in powders can be referred to as humidity caking. The degree of hygroscopicity varies among dairy powders. It has been reported that the crystalline sugars are less hygroscopic when compared to their amorphous counterparts (Bronlund and Paterson. 2004). Humidity caking is generally caused by wetting on the surface of the particles and dissolution may also occur sometimes (Rogé and Mathlouthi, 2003). This dissolution when crystalline material absorbs water and turns into a solution is called deliquescence (Martin, 2000). Deliquescence generally occurs at a relative humidity that is

characteristic of the crystalline solid and is a first-order phase transition (Mauer and Taylor, 2010). The schematic diagram representing different stages in a deliquescence process is shown in Figure 2.4. The presence of residual moisture in dairy powders can lead to significant changes in the physical and chemical properties of the powder. It has been reported that there are five mechanisms by which water-solid interactions can occur and these include adsorption of water on the solid particle surface, capillary condensation, deliquescence, crystal hydrate formation, and vapor absorption into the bulk of amorphous solids (Mauer and Taylor, 2010).



**Figure 2.4** Schematic of the deliquescence process (Adapted from Mauer and Taylor, 2010).

When the deliquescence relative humidity is greater than the RH of the environment, usually water gets adsorbed to a few molecular layers on the surface of the solid by the mechanism of adsorption. However, when the RH increases, more vapor gets adsorbed at the surface to the point where a narrow layer of saturated solution form on the particle when the RH of the environment is greater than the deliquescence relative humidity. This layer is formed as a vapor pressure lower than that of pure water (Zografis, 1988). Since water in the layer formed has less thermodynamic activity, there is a driving force that exists for condensation of water when the vapor pressure exceeds the vapor pressure of the saturated solution layer, or when the RH of the surroundings is greater than the deliquescence relative humidity. The capillary condensation occurs in lactose when the relative humidity is greater than 80% (Bronlund and Paterson, 2004).

Lactose being water-soluble, the capillary condensation may lead to dissolution causing deliquescence. The water vapor condensing into the saturated layer causes an increase in the vapor pressure of the layer to that of surrounding water vapor pressure. Then the dissolution of solid occurs until saturation is attained which reduces the vapor pressure back to the deliquescence relative humidity. Thus, condensation and dissolution occur alternately until the solid completely dissolves (Van Campen et al., 1983).

Kevin equation gives the relation between vapor pressure required for condensation to occur to the size of the capillary, the Kevin radius ( $r_k$ ) [m], the wetting angle ( $\theta$ ) and surface tension ( $\sigma$ ) and thus can be used to predict the Kevin radius where capillary condensation occurs for different water activities.

$$a_w = \frac{P_v}{P_w} = e^{\left(\frac{-2\sigma\cos\theta V_0}{r_k R(T+273.15)}\right)}$$

where  $a_w$  is water activity,  $P_v$  is the water vapor pressure of the material [Pa],  $P_w$  is the vapor pressure of pure water [Pa] and  $V_0$  is the volume of saturated sucrose solution occupies [ $\text{m}^3/\text{mol}$ ]. It has been reported that from a water activity between 0.75 and 0.8, the capillary radius increases exponentially indicating that significant liquid bridges could be formed between particles from this point (Billings et al., 2006).

Partial deliquescence can occur when, the saturated solution layer formed on the surface of the particle forms liquid bridges if the relative humidity of the environment is greater than the deliquescence relative humidity (Mauer and Taylor, 2010; Rogé and Mathlouthi, 2003). However, crystallization can occur from the saturated solution with the release of water when the RH of the environment is lower than the efflorescence relative humidity (Carpin et al., 2017). When moisture is removed from the formed liquid bridge due to effects such as temperature change then the solid bridge can be formed between particles (Billings et al., 2006).

Additionally, it has been reported that even without a reduction in RH solid bridges can be formed by mass transfer of particles to bridges with storage time (Kirsch et al., 2011; Scholl and Schmidt, 2014).

When mixes containing different ingredients have different deliquescence then it can lead to a phenomenon called deliquescence lowering. Adams and Merz (1929) were the first who reported on deliquescence lowering in fertilizers by measuring the vapor pressures above saturated solutions. Yamamoto and Takahashi (1952) reported deliquescence lowering in pharmaceutical sugars and observed that the deliquescence relative humidity ( $RH_0$ ) of a mixture of glucose, sucrose, and citric acid was more or less equal to the product of the  $RH_0$ s of the individual constituents, and thereby the mixture had a lower  $RH_0$  (called  $RH_{0mix}$ ). It has been reported that the  $RH_0$  of a compound could vary with varying temperatures. It has been found that with the increase in temperature the solubility also increases, and thus decrease  $RH_0$  (Mauer and Taylor, 2010).

In food formulations or ingredients and food products, several ingredients could be deliquescent. When deliquescence occurs, other components which are not deliquescent gets exposed to the solvent environment. This will lead to increased moisture sensitivity thus causing problems even at lower RHs. Deliquescence can be prevented by storing the powders below the critical RH that would prevent deliquescence. Although, the control of RH can be difficult as the water is ubiquitous in the environment and changes in temperature could aggravate the problems relating to deliquescence (Mauer and Taylor, 2010).

### **Mechanical caking**

Mechanical caking can be defined as the caking due to powder compression from mechanical pressure. It has been reported that mechanical caking can be an exacerbating factor

that intensifies amorphous and humidity caking (Carpin et al., 2017). Mechanical caking is caused when particles come very close to each other due to decreased interparticle distance. The strongest interaction in dry powder is Van der Waals forces and this is observed to be intense when interparticle distances are short. It is important to understand more about consolidation stress and its effects on mechanical caking. Jenike (1964) observed that when powders are exposed to compression/consolidation stress it can cause flowability issues, but it also depends on the physical properties of the powder and some environmental conditions like temperature. When powder undergoes compression stress it becomes compact and thus the interparticle distance decreases. As the interparticle distance decreases, there is an increased contact region between particles due to the rearrangement of particles due to compression and plastic deformation. Thus, the cohesion between particles increases and becomes greater with time (Malby et al., 1993). Caking can thus also occur in a dry environment due to mechanical pressure. It is not always necessary that caking requires the presence of moisture. Thus, it is not necessary that caking will occur due to mechanical pressure. There are other factors such as ambient conditions and particle properties. It is very important to understand the reasons behind caking so that it can be easier to characterize and control the caking of powders.

### **Maillard browning**

The Maillard reaction is a type of non-enzymatic browning reaction between a reducing sugar like lactose or glucose, with a compound having a free amino group and is named after a French chemist Louis Camille Maillard (Maillard, 1912). In food systems, the Maillard reaction mostly occurs between essential amino acid lysine (as it has a free amino group) which reacts with simple carbohydrates. Various other food components such as free amino acids, proteins, peptides, and vitamins take part in non-enzymatic browning reactions in the presence of sugars

or, other carbonyls (Friedman, 1996). It is very important to discuss here as lactose is a reducing sugar that takes part in the Maillard reaction which affects the quality of various lactose rich powders.

Generally, the Maillard reaction can be divided into three stages which include the early Maillard reaction, the advanced Maillard reaction and the final Maillard reaction (Mauron, 1981; Perez-Locas and Yaylayan, 2010). In the early stage of the Maillard reaction, the condensation of the reducing sugar with the amino group results in the formation of a Schiff's base and the Amadori rearrangement product. In the advanced stages of the Maillard reaction, this Amadori rearrangement product breaks into numerous fission products of the sugar-amino compound. In the final stages of Maillard reaction the condensation of amino compounds and sugar fragments into the formation of brown pigments, known as the melanoidins (Van Boekel, 1998).

### **Chemistry of Maillard reaction**

In the early stages of the Maillard reaction, a reducing sugar condenses with a free amino group possessing compound leading to the formation of a condensation product, N-substituted glucosamines, that rearranges to form the Amadori rearrangement product (ARP) but, the further degradation of the ARP is pH-dependent. When the pH of the system is equal to or lower than 7, it forms furfural (involving pentoses) or hydroxymethylfurfural (HMF) (involving hexoses) through 1,2-enolisation. Although, when the pH is greater than 7 it undergoes 2,3-enolisation forming reductones such as 4-hydroxy-5-methyl-2,3-dihydrofurfuran-3-one and some fission products (acetol, diacetyl, pyruvaldehyde). The carbonyl groups possessing free amino group reacts which incorporates nitrogen to reaction products. The dicarbonyl compounds further react with amino acids forming aldehydes and  $\alpha$ -aminoketones and is called as Strecker degradation. This Strecker degradation is important in yielding aroma-active aldehydes and pyrazine



derivatives through the reaction between amino acids and  $\alpha$ -dicarbonyls (Perez-Locas and Yaylayan, 2010). In the final stage, there are various reactions such as dehydrations, cyclizations, retraldolisations, isomerizations, rearrangements and condensations, which results in the formation of a brown pigment called the melanoidins (Martins et al., 2000). These melanoidins are responsible for the dark brown color in many foods and are known to be a contributing factor of non-enzymatic browning. It is very important to know in detail about the chemistry and characterization of Maillard reaction as it will give insights into the nutritional, sensory, and quality deterioration.

### **Factors affecting Maillard reaction**

There are various factors that affect the Maillard reaction in foods such as the water activity ( $a_w$ ), pH, concentration and type of reactant species. Also, various external factors affect Maillard browning such as temperature and RH during storage. Now, let's have a look at the effect of these factors on Maillard browning in detail.

#### **Effect of reactant species**

The Maillard reaction is affected by the amount and type of the initial reactant species. The rate of the Maillard reaction is also affected by the amount of reducing sugar available for reaction. It was observed that the fructose browned faster than glucose at low concentrations of glycine, whereas, at high amino acid concentration, the reverse occurred (Kato et al., 1969). Jing and Kitts (2002) reported that the Maillard reaction of glucose, fructose, and ribose with casein was observed at 55°C, pH 7.0 for up to 28 days and the browning was found to increase with increasing heating time. Additionally, it was reported that the browning development was higher for the glucose–casein mixture than that of the fructose–casein model after 19 days. Different sugars have different reaction rates that result in the formation of Maillard reaction products

(MRPs) in the Maillard reaction (Wijewickreme et al., 1999; Brands et al., 2000). Hexoses react less rapidly than pentoses. The increasing order of ring-opening and browning among the hexoses are D-glucose, D-mannose, and D-galactose (Hodge and Osman, 1976). Although, hexoses are more reactive than D-fructose (Spark, 1969). A non-reducing disaccharide such as sucrose can also participate in the Maillard reaction if hydrolyzed by acid or enzyme action during storage into a reducing moiety (Karel and Labuza, 1969). Sucrose will take part in Maillard reaction only if the glycosidic bond is hydrolyzed which could be facilitated by a low pH, in turn, increasing the rate of Maillard reaction (Gogus et al., 2010). It has been reported that glucose browns at a much faster rate than fructose (Patel et al., 2013). Dattatreya et al. (2010) reported that the sweet whey powder samples which contain galactose (1.5 g/L) have a greater degree of browning when compared to those containing just lactose (3.0 g/L) indicating that residual sugars have a significant effect on the browning. The reason being that the presence of sugars such as galactose could increase the initial rate of reducing sugar- dependent browning when it reacts with a given amino compound primarily by increasing the rate at which the sugar's ring opens to a reactive, reducible form (Overend et al., 1961; Dattatreya et al., 2010). The preferential site for initiation of the Maillard reaction is generally the amino group of lysine during the storage of foods containing protein and carbohydrates. The amount of lysine available for reaction determines the rate of protein quality losses (Saltmarch and Labuza, 1982; Ajandouz et al., 2001). The nonenzymatic browning reactions of fructose and fructose-lysine aqueous model systems were investigated at 100°C between pH 4.0 and pH 12.0 by measuring the loss of reactants and monitoring the pattern of UV-absorbance and brown color development. At all the pH values tested, the loss of fructose was lower in the presence than in the absence of lysine.

And, in lysine-containing fructose solution, the sugar disappeared more rapidly than the amino acid.

### **Effect of pH**

Several authors have reported that the Maillard reaction increases as pH increases. Wolfrom et al. (1974) reported that both the buffering capacity and initial pH of a product influence the rate of Maillard reaction. It has been also reported that as the Maillard reaction proceeds, the pH of the system falls which could be attributed to the decrease in free amino groups in the system (Saltmarch and Labuza, 1982). Moreover, pH is known to affect the pathway undertaken towards melanoidin formation. Amadori compounds will degrade and polymerize via 1,2-eneaminol formation producing furfurals at acidic conditions. In contrast, it favors 2,3-enediol pathway generating a greater number of reductones, dicarbonyls, aldehydes, furaneols, pyrones, and other fission products at alkaline conditions (Ames, 1990). These compounds (dicarbonyls and aldehydes) formed are ideal for Strecker degradation reactions to take place which generate numerous volatiles thus contributing to the organoleptic properties (Perez-Locas and Yaylayan, 2010).

### **Effect of temperature**

Increasing temperature is known to increase the rate of Maillard reaction. Saltmarch and Labuza (1982) reported that in a lysine model system the brown pigment formation was not visibly detected after 20 days of storage at 25°C however it was visible after 80 days of storage. Patel et al. (2013) reported that in foods that contain fructose, the increase in Maillard browning maybe 5-10 times for an increase of each 10°C. It has also been reported that the browning rate increases with unsteady state conditions when compared to steady-state conditions (Saltmarch

and Labuza, 1982). Most of the studies conducted are under constant and controlled temperatures which is not realistic in commercial powder/food storage.

### **Effect of water activity**

The  $a_w$  of powders/food is influenced by the RH of the surrounding environment. Most of the deteriorative reactions increase as  $a_w$  increase above the monolayer moisture content. Labuza and Saltmarch (1982) reported that maximum browning happens at the  $a_w$  range of 0.50 to 0.75. Also, with the increase in  $a_w$  from 0.33 to 0.54 the browning rate increases and then plateaus above that (Bell, 1995). At lower  $a_w$  values, the browning reactions tend to proceed slowly which could be due to increasing diffusion resistance due to high viscosity. Also, at lower  $a_w$  values, water is chemically adsorbed to polar sites on the surface of the food product making it unavailable for many chemical reactions to occur. At higher  $a_w$  values, the rate of browning reaction again slows down, because of the dilution of the reactants (Patel et al., 2013). In dehydrated skim milk at 54°C, the rate of browning decreases with decreasing relative humidity and the formation of brown products is the maximum at 75% relative humidity (Labuza et al., 1992).

### **Measures to control browning**

It is important to control or reduce the Maillard reaction as it could cause deterioration of powders/foods. The various ways by which Maillard browning can be decreased or inhibited include:

- Controlling factors such as temperature, storage time, and relative humidity
- Controlling parameters such as  $a_w$  and pH
- Reduction of reducing sugar content/ amino acid content
- Use of chemical inhibitors

## References

- Adams, J. R., and A. R. Merz. 1929. Hygroscopicity of fertilizer materials and mixtures. *Ind. Eng. Chem.* 21: 305-307.
- Adhikari, B., T. Howes, B. Bhandari, and V. Truong. 2001. Stickiness in foods: A review of mechanisms and test methods. *Int. J. Food Prop.* 4:1-33.
- Aguilera, J., J. del Valle, and M. Karel. 1995. Caking phenomena in amorphous food powders. *Trends Food Sci. Technol.* 6:149-155.
- Ajandouz, E. H., L. S. Tchiakpe, F. D. Ore, A. Benajiba, and A. Puigserver. 2001. Effect of pH on caramelization and Maillard reaction kinetics in fructose–lysine model systems. *J. Food Sci.*, 66:926-931.
- Ames, J., R. J. Bailey, and J. Mann. 1998. Recent advances in the analysis of colored Maillard reaction products, In *The Maillard Reaction in Foods and Medicine*, London: Royal Society of Chemistry. 76-82.
- Bell, L. N. 1995. Kinetics of non-enzymatic browning in amorphous solid systems: distinguishing the effects of water activity and the glass transition. *Food Res. Int.* 28:591-597.
- Bhargava, A., and P. Jelen. 1996. Lactose solubility and crystal growth as affected by mineral impurities. *J. Food Sci.* 61:180-184.
- Billings, S. W., Bronlund, J. E., and Paterson, A. H. J. 2006. Effects of capillary condensation on the caking of bulk sucrose. *J. Food Eng.* 77: 887-895.
- Brands, C. M., G. M. Alink, M. A. van Boekel and W. M. Jongen. 2000. Mutagenicity of heated sugar– casein systems: Effect of the Maillard reaction. *J. Agric. Food Chem.* 48:2271-2275.
- Brennan, J. G., J. Herrera, and R. Jowitt. 1971. A study of some of the factors affecting the spray drying of concentrated orange juice on a laboratory scale. *J Food Technol.* 6:295-307.
- Bronlund, J., and T. Paterson. 2004. Moisture sorption isotherms for crystalline, amorphous and predominantly crystalline lactose powders. *Int. Dairy J.* 14:247-254.
- Bushill, J. H., W. B. Wright, C.H.F. Fuller, and A. V. Bell. 1965. The crystallisation of lactose with particular reference to its occurrence in milk powder. *J. Sci. Food Agric.* 16:622.
- Carpin, M., H. Bertelsen, A. Dalberg, J. K. Bech, J. Risbo, P. Schuck, and R. Jeantet. 2017. How does particle size influence caking in lactose powder. *J. Food Eng.* 209: 61-67.
- Carpin, M., H. Bertelsen, J. K. Bech, R. Jeantet, J. Risbo, and P. Schuck. 2016. Caking of lactose: A critical review. *Trends Food Sci. Technol.* 53:1-12.

- Chandrapala, J., R. Wijayasinghe, and T. Vasiljevic. 2016. Lactose crystallization as affected by presence of lactic acid and calcium in model lactose systems. *J. Food Eng.* 178:181-189.
- Chuy, L. E., and T. P. Labuza. 1994. Caking and stickiness of dairy-based food powders as related to glass transition. *J. Food Sci.* 59:43-46.
- Dattatreya, A., W. Lee and S. A. Rankin. 2010. Presence of galactose and glucose promotes browning of sweet whey powder. *J. Dairy Sci.* 93:2354-2357.
- Descamps, N., and Palzer, S. 2007. Modeling the sintering of water soluble amorphous particles. PARTEC, Nuremburg, Germany.
- Downton, G. E., J. L. Flores-Luna, and C. J. King. 1982. Mechanism of stickiness in hygroscopic, amorphous powders. *Ind. Eng. Chem. Fund.* 21:447-451.
- Fernández, E., C. Schebor, and J. Chirife. 2003. Glass transition temperature of regular and lactose hydrolyzed milk powders. *LWT-Food Sci. Technol.* 36:547-551.
- Fitzpatrick, J. J., M. Hodnett, M. Twomey, P. Cerqueira, J. O'flynn, and Y. H. Roos. 2007. Glass transition and the flowability and caking of powders containing amorphous lactose. *Powder Technol.* 178:119-128.
- Foster, K. D., J. E. Bronlund, and A. H. J. Paterson (Tony). 2006. Glass transition related cohesion of amorphous sugar powders. *J. Food Eng.* 77:997-1006.
- Frenkel, J., 1945. Viscous flow of crystalline bodies under action of surface tension. *J. Phys.* 9: 385-391.
- Friedman, M. 1996. Food browning and its prevention: an overview. *J. Agric Food Chem.* 44:631-653.
- Gogus, F., S. Fadıloglu, and S. Cigdem. 2010. Biological Oxidations: Enzymatic and Nonenzymatic Browning Reactions and Control Mechanisms, In: *Advances in Food Biochemistry*, Yildiz F (Ed.). CRC Press, UK, 341-382.
- Haase, G., and T. A. Nickerson. 1966. Kinetic reactions of alpha and beta lactose. I. Mutarotation. *J. Dairy Sci.* 49:127-132.
- Haque, M. K., and Y. H. Roos. 2004. Water plasticization and crystallization of lactose in spray-dried lactose/protein mixtures. *J. Food Sci.* 69:23-29.
- Hargrove, R. E., F. E. McDonough, D. E. Lacroix and J. A. Alford. 1976. Production and properties of deproteinized whey powders. *J. Dairy Sci.* 59:25-33.
- Harju, M. 2001. Milk sugars and minerals as ingredients. *Int. J. Dairy Technol.* 54:61-63.
- Hartmann, M. and S. Palzer. 2011. Caking of amorphous powders—Material aspects, modelling and applications. *Powder Technol.* 206:112-121.

- Hodge, J. E., and Osman, E. M. 1976. Carbohydrates. In Food Chemistry, Part 1. Fennema, O. R., Ed. New York, Marcel Dekker.
- Holsinger, V. 1997. Physical and chemical properties of lactose. Advanced Dairy Chemistry. Vol. 3. P. F. Fox, ed. Springer, New York, NY.
- Holsinger, V.H., 1988. Lactose. In: Wong, N.P., Jenness, R., Keeney, M., Marth, E.H. (Eds.), Fundamentals of Dairy Chemistry, third ed. Van Nostrand Reinhold, New York, 279-342.
- Hourigan, J. A., E. V. Lifran, L. T., Y. Listiohadi and R. W. Sleight. 2013. Lactose: Chemistry, processing, and utilization. Advances in Dairy Ingredients. 21-41.
- Hudson, C. S. 1904. The hydration of milk sugar in solution. J. Am. Chem. Soc. 26:1065-1082.
- Huppertz, T., and I. Gazi. 2016. Lactose in dairy ingredients: Effect on processing and storage stability. J. Dairy Sci. 99:6842-6851.
- Ibach, A., and M. Kind. 2007. Crystallization kinetics of amorphous lactose, whey-permeate and whey powders. Carbohydr. Res. 342:1357-1365.
- Jelen, P., and S.T. Coulter, 1973. Effects of certain salts and other whey substances on the growth of lactose crystals. J. Food Sci. 38:1186-1189.
- Jenike, A. W. 1964. Storage and flow of solids. Bulletin of the University of Utah, 53 and 26 (Bull No. 123 of Utah engineering experiment station, eleventh printing (1986), revised in 1980).
- Jing, H., and D. D. Kitts. 2002. Chemical and biochemical properties of casein–sugar Maillard reaction products. Food Chem. Toxicol. 40:1007-1015.
- Jouppila, K., and Y. H. Roos. 1994a. Water sorption and time-dependent phenomena of milk powders. J. Dairy Sci. 77:1798-1808.
- Jouppila, K., and Y. H. Roos. 1994b. Glass transitions and crystallization in milk powders. J. Dairy Sci. 77:2907-2915.
- Jouppila, K., J. Kansikas and Y. H. Roos. 1997. Glass transition, water plasticization, and lactose crystallization in skim milk powder. J. Dairy Sci. 80:3152-3160.
- Karel, M., and Labuza, T. P. 1969. Non-enzymatic browning in model systems containing sucrose. J. Agric. Food Chem. 76:717-19,
- Kato, H., M. Yamamoto, and M. Fujimaki. 1969. Mechanism of browning degradation of D-fructose in special comparison with D glucose– glycine reaction. Agric. Biol. Chem. 33:939-948.
- Kelly, P. M. 2009. Significance of Lactose in Milk Powders. Pages 80-97 in Advanced Dairy Chemistry. Vol. 3. P. McSweeney and P. F. Fox, ed. Springer, New York, NY.

- Kirsch, R. M., R. A. Williams, U. Bröckel, R. B. Hammond, and X. Jia. 2011. Direct observation of the dynamics of bridge formation between urea prills. *Ind. Eng. Chem. Res.* 50:11728-11733.
- Labuza, T. P. 1992. The kinetics of nonenzymatic browning. In *Physical chemistry of foods* Schwarzberg H G and Hartel R W (Eds.), Marcel Dekker, Inc., New York.
- Labuza, T. P., and M. Saltmarch. 1982. Kinetics of browning and protein quality loss in whey powders during steady state and nonsteady state storage conditions. *J. Food Sci.* 47:92-96.
- Lai, H.-M., and S. J. Schmidt. 1990. Lactose crystallization in skim milk powder observed by hydrodynamic equilibria, scanning electron microscopy and <sup>2</sup>H nuclear magnetic resonance. *J. Food Sci.* 55:994-999.
- Lazar, M. E., A. H. Brown, G. S. Smith, F. F. Wong, and F. E. Lindquist. 1956. Experimental production of tomato powder by spray drying. *Food Technol.* 10:129-134.
- Lerk, C. F. 1993. Consolidation and compaction of lactose. *Drug Dev. Ind. Pharm.* 19:2359-2398.
- Listiohadi, Y. D., J. A. Hourigan, R. W. Sleight and R. J. Steele. 2005. Effect of milling on the caking behaviour of lactose. *Aust. J. Dairy Technol.* 60:214.
- Listiohadi, Y., J. A. Hourigan, R. W. Sleight and R. J. Steele. 2008. Moisture sorption, compressibility and caking of lactose polymorphs. *Int. J. Pharm.* 359:123-134.
- Maillard, L. C. 1912. Action des acides amines sur les sucres; formation des melanoidines par voie methodique. *Comptes R. Acad. Sci.* 154:66-68.
- Malby, L. P., G. G. Enstad, and S. R. De Silva. 1993. Investigation of the behaviour of powders under and after consolidation. In *Proceedings of symposium (Reliable flow of particulate solids II)*. Oslo, Norway: EFCHE publication No. 96, 23<sup>rd</sup>-25<sup>th</sup> August.
- Martin, S. T. 2000. Phase transitions of aqueous atmospheric particles. *Chem. Rev.* 100:3403-3454.
- Martins, S. I., W. M. Jongen, and M. A. Van Boekel. 2000. A review of Maillard reaction in food and implications to kinetic modelling. *Trends Food Sci. Technol.* 11:364-373.
- Mauer, L. J. and L. S. Taylor. 2010. Water-solids interactions: Deliquescence. *Annu. Rev. Food Sci. Technol.* 1:41-63.
- Mauron, J. 1981. The Maillard reaction in food: A critical review from the nutritional standpoint. *Prog Food Nutr Sci.* 5:5-35.



- McLeod, J. 2007. Nucleation and Growth of Alpha Lactose Monohydrate: A Thesis Presented in Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy in Process Engineering at Massey University.
- Mimouni, A., P. Schuck, and S. Bouhallab. 2005. Kinetics of lactose crystallization and crystal size as monitored by refractometry and laser light scattering: Effect of proteins. *Le Lait*. 85:253-260.
- Modler H. W., and L. P. Lefkovitch. 1986. Influence of pH, casein, and whey protein denaturation on the composition, crystal size, and yield of lactose from condensed whey. *J Dairy Sci*. 69:684-97.
- Nijdam, J., A. Ibach, and M. Kind. 2008. Fluidisation of whey powders above the glass-transition temperature. *Powder Technol*. 187:53-61.
- O'Callaghan, D., and S. Hogan. 2013. The physical nature of stickiness in the spray drying of dairy products: A review. *Dairy Sci. Technol*. 93:331-346.
- Overend, W. G., A. R. Peacocke, and J. B. Smith. 1961. Reactions at position 1 of carbohydrates. part I. the polarographic reduction of carbohydrates. *J. Chem. Soc*. 3487-3497.
- Palzer, S. 2005. The effect of glass transition on the desired and undesired agglomeration of amorphous food powders. *Chem. Eng. Sci*. 60:3959-3968.
- Patel, K. N., R. B. Modi, H. G. Patel, and K. D. Aparnathi. 2013. Browning, its chemistry and implications in dairy products—A review. *Indo-Am. J Agric. Vet. Sci*. 1:1-12.
- Paterson, A. 2009. Production and uses of lactose. *Advanced Dairy Chemistry*. Vol. Volume 3. P. L. H. McSweeney and P. F. Fox, ed. Springer, New York, NY. 105-120.
- Paterson, A., G. Ripberger, and R. Bridges. 2015. Measurement of the viscosity of freeze dried amorphous lactose near the glass transition temperature. *Int. Dairy J*. 43:27-32.
- Peleg, M. 1977. Flowability of food powders and methods for its evaluation—A review. *J. Food Process Eng*. 1:303-328.
- Perez-Locas, C., and V. A. Yaylayan. 2010. The Maillard reaction and food quality deterioration. *Chemical Deterioration and Physical Instability of Food and Beverages*. 70-94.
- Renzetti, S., J. A. Voogt, L. Oliver, and M. B. J. Meinders. 2012. Water migration mechanisms in amorphous powder material and related agglomeration propensity. *J Food Eng*. 110:160-168.
- Rogé, B., and M. Mathlouthi. 2003. Caking of white crystalline sugar. *Int. Sugar J*. 105:128-130.
- Roos, Y. 2003. Thermal analysis, state transitions and food quality. *J Therm. Anal. Calorim*. 71:197-203.

- Roos, Y. H. 2002. Importance of glass transition and water activity to spray drying and stability of dairy powders. *Le Lait*. 82:475-484.
- Roos, Y. H. 2010. Glass transition temperature and its relevance in food processing. *Annu. Rev. Food Sci. Technol.* 1:469-496.
- Roos, Y. H., and Karel, M. 1990. Differential scanning calorimetry study of phase transitions affecting the quality of dehydrated materials. *Biotechnol. Prog.* 6:159-163.
- Roos, Y., and M. Karel. 1991. Plasticizing effect of water on thermal behavior and crystallization of amorphous food models. *J. Food Sci.* 56:38-43.
- Roos, Y., and M. Karel. 1992. Crystallization of amorphous lactose. *J. Food Sci.* 57:775-777.
- Roos, Y., and M. Karel. 1993. Effects of glass transitions on dynamic phenomena in sugar containing food systems. *The Glassy State in Foods*. 207-222.
- Roth, D. 1976. Amorphous icing sugar produced during crushing and recrystallisation as the cause of agglomeration and procedures for its avoidance. Ph.D. thesis, University of Karlsruhe, Karlsruhe (in German).
- Saltmarch, M., and T. P. Labuza. 1982. Nonenzymatic browning via the Maillard reaction in foods. *Diabetes*. 31:29-36.
- Scholl, S. K., and S. J. Schmidt. 2014. Determining the physical stability and water-soluble interactions responsible for caking during storage of anhydrous glucose. *J Food Meas. Charact.* 8:326-335.
- Shrestha, A. K., T. Howes, B. P. Adhikari, and B. R. Bhandari. 2007. Water sorption and glass transition properties of spray dried lactose hydrolysed skim milk powder. *LWT-Food Sci. Technol.* 40:1593-1600.
- Smart, J. B., and J. M. Smith. 1992. Effect of selected compounds on the rate of  $\alpha$ -lactose monohydrate crystallization, crystal yield and quality. *Int. Dairy J.* 2:41-53.
- Smith, K. 2017. *Dried Dairy Ingredients*. Wisconsin Center for Dairy Research.
- Spark, A. A. 1969. Role of amino acids in non-enzymatic browning. *J. Sci. Food Agric.* 20:308-16.
- Thomas, M., J. Scher, and S. Desobry. 2004. Lactose/ $\beta$ -lactoglobulin interaction during storage of model whey powders. *J. Dairy Sci.* 87:1158-1166.
- Troy, H. C., and P. F. Sharp. 1930.  $\alpha$  and  $\beta$  lactose in some milk products. *J. Dairy Sci.* 13:140-157.
- Van Boekel, M. 1998. Effect of heating on maillard reactions in milk. *Food Chem.* 62:403-414.

- Van Campen L., G. L. Amidon, G. Zografi. 1983. Moisture sorption kinetics for water-soluble substances. I: Theoretical considerations of heat transport control. *J. Pharm. Sci.* 72:1381-88
- Vu, L. T., L. Huynh and J. A. Hourigan. 2009. Effects of solvents on characteristics of crystalline lactose extracted in ternary and quaternary systems. *Adv. Powder Technol.* 20:251-256.
- Walstra, P., and R. Jenness. 1984. *Dairy Chemistry and Physics*. John Wiley and Sons, Hoboken, NJ.
- Walstra, P., J. T. Wouters, and T. J. Geurts. 2005. *Dairy Science and Technology*. Second ed. CRC Press, Boca Raton, FL.
- Wijewickreme, A. N., Z. Krejpcio and D. D. Kitts. 1999. Hydroxyl scavenging activity of glucose, fructose, and ribose-lysine model Maillard products. *J. Food Sci.* 64:457-461.
- Wolfrom, M. L., Naoki K and Derek H. 1974. Factors affecting the Maillard reaction between the sugars and amino acids studies on the nonenzymic browning of dehydrated orange juice. *J. Agric. Food Chem.*, 22: 796.
- Wong, S. Y., and R. W. Hartel. 2014. Crystallization in lactose refining—A review. *J. Food Sci.* 79: R272.
- Wong, S. Y., R. K. Bund, R. K. Connelly and R. W. Hartel. 2011. Determination of the dynamic metastable limit for  $\alpha$ -lactose monohydrate crystallization. *Int. Dairy J.* 21:839-847.
- Yamamoto, and T. Takahashi. 1952. Studies on the hygroscopicity and moisture barriers of medicines. *Ann. Repts. Shionogi Res. Lab.* 1:142-147.
- Zografi G. 1988. States of water associated with solids. *Drug Dev. Ind. Pharm.* 14:1905-26.

## **Chapter 3 - Research Objectives**

This study focused on evaluating the influence of lactic acid and galactose on the storage stability of high lactose dairy powders. The specific objectives of the study are:

1. To study the effect of lactic acid and galactose on the physiochemical and thermal properties of deproteinized whey powders during storage.
2. To study the effect of lactic acid, galactose, and storage temperature on the physiochemical and thermal properties of milk permeate powders during storage.

# **Chapter 4 - Effect of varying levels of lactic acid and galactose on the physiochemical and thermal properties of deproteinized whey powders during storage**

## **Abstract**

Deproteinized whey (DPW) is obtained as a permeate fraction during the ultrafiltration of whey and generally contains a minimum of 76% (db) lactose. It is further processed into a non-hygroscopic and free-flowing DPW powder by crystallization and spray drying. The DPW powders being high in lactose may undergo changes such as caking and Maillard browning during storage. This study aimed at investigating the effect of different levels of lactic acid and galactose on the physical, chemical, and thermal properties of DPW powders. Two lots of concentrated permeate with total solids of ~77% were procured from a commercial manufacturer. The total solids were adjusted to 45% with water and were divided into 9 subsamples with treatment combinations of high, medium, low levels of lactic acid by adjusting the pH to 5.0 by adding 0.2% lactic acid, pH 5.55 with no addition, and pH 6.10 by adding 2% NaOH and galactose (0, 0.15, and 0.30%) as per the experimental design. The DPW powders were spray dried in a lab-scale spray dryer with an inlet temperature of 180°C and outlet temperature 83-88°C. The spray dried DPW powders were sealed and stored at 25°C for 0 (control), 30, 60, 90, 150, 210, and 270 days. At each data point, the powders were analyzed for water activity ( $a_w$ ), color, browning index (BI), FAST index (FI), hygroscopicity, caking, differential scanning calorimetry (DSC), and microstructure. The  $a_w$ , color values, and BI of the powders immediately after spray drying were not significantly different ( $p > 0.05$ ). In the DPW powders during storage, the main effects lactic acid and the interaction term lactic acid x

galactose were found to have significant ( $p < 0.05$ ) effect on  $L^*$  values, hygroscopicity, caking, and FI. On storage, the time and the interaction effects time x galactose, time x lactic acid x galactose each were also found to have a significant ( $p < 0.05$ ) effect on  $a_w$ ,  $L^*$  values, and FI. The thermal properties of the stored DPW powders showed a dehydration endothermic peak at around  $142^\circ\text{C}$ , indicating the loss of crystalline water. Overall, the DPW powders exhibited significant difference for the treatment combinations and the storage changes in DPW powders can be modulated by a concomitant control on the amount of lactic acid and galactose levels.

Key Words: Deproteinized whey powder, lactic acid, galactose

## **Introduction**

The popularity and the increasing demand for high protein foods have driven manufacturers to develop new and innovative high protein beverages, protein bars, etc., which in turn has resulted in the increased production of whey. The urge for whey utilization has increased as whey disposal poses a threat to the environment due to the high biochemical oxygen demand of whey (Hargrove et al., 1976). Thus, the researchers and manufacturers are finding different ways to utilize whey which is rich in lactose. Although, due to the ongoing rise in cheese production and protein concentrates manufacture, a large amount of whey and whey permeate still exists. Whey was traditionally regarded as a low valued product however currently it has been used to manufacture various dry ingredients with high value (Carpin et al., 2017). Deproteinized whey powder (DPW) is a coproduct obtained from the ultrafiltration of whey which is widely used in the baking industry due to the presence of high amounts of lactose. It generally consists of 76-85% lactose, 2-7% protein, 3-4.5% moisture, 0-0.1% fat, and 8-11% ash. Many uses have been found for DPW powder such as application in bakery products, dry mixes, snack foods, confections, and animal feed. Advantages of DPW powders include non-

hygroscopic, free flowing, complete soluble powder with dairy flavor and has been used as economical whey lactose replacer.

The presence of lactose in dairy powders can adversely affect powder properties which can cause some undesirable changes such as, stickiness and caking during storage. Caking can happen at any point during manufacture, transportation or during storage and can be attributed to different factors. Also, lactose being a reducing sugar could take part in Maillard reactions which could cause browning in dairy powders. There are various factors that attribute to this such as the state of lactose, storage temperature, storage time, relative humidity, etc. Therefore, it is very critical to understand the processing conditions and storage changes happening to the powders which help in handling, processing, and storage of the lactose rich powders.

The handling and storage of high lactose powders can be a hassle to the food industry due to the undesirable agglomeration of powder particles, referred to as caking which in turn results in economic loss. Moreover, caked powder results in extensive processing times and decreased product quality. The presence of amorphous material and other impurities must be evaluated in crystalline lactose powders, as they can cause amorphous and humidity caking. Caking mechanisms in food powders have been reviewed previously (Carpin et al., 2016).

Understanding the production process and storage conditions is the key to prevent caking. The presence of impurities can increase the tendency of caking in high lactose powders. Therefore, it is crucial to control the washing and purification process in manufacturing to prevent caking in the final product. Carpin et al. (2017) reported that the presence of impurities in lactose powder enhances moisture sorption and caking. Indeed, any impurity (peptides or minerals) in higher concentrations on the surface of the crystals can also lead to enhanced sorption behavior which

can be detrimental to the powder quality. It has been also reported that galactose and glucose can accelerate the browning of sweet whey powder (Dattatreya et al., 2010).

Changes in the storage stability of DPW powders due to varying levels of lactic acid and galactose have not been studied previously. The objective of this study deals with the effect of the presence of impurities (lactic acid and galactose) on the storage stability of DPW powders.

## **Materials and Methods**

### **Experimental design**

The deproteinized whey permeate (2 lots) with approximately 77% total solids was collected from a commercial manufacturer within the United States. The total solids were adjusted to 45% by adding water and samples were divided into 9 subsamples (Table 4.1) with varying levels of lactic acid and galactose. The treatment combinations were such that the high lactic acid (by adjusting pH to 5 with lactic acid), medium lactic acid with pH 5.55 (no addition) and low lactic acid (by adjusting pH to 6.10 with NaOH) and galactose (0, 0.15, and 0.30%) as per the experimental design. The treatment with low lactic acid (pH 6.1) low galactose (0%) is considered as control. Each of the samples were spray dried and stored in poly-lined kraft bags (Proliant Dairy, USA) and were stored at 25°C in an incubator (Hettcube 200R, MA, USA) for 0 (control), 30, 60, 90, 150, 210, and 270 days. At each data point, the powders were analyzed for water activity ( $a_w$ ), color, browning index (BI), fluorescence of advanced Maillard products and soluble tryptphan index (FI), hygroscopicity, caking, differential scanning calorimetry (DSC), and microstructure. All the measurements were carried out in duplicates.

### **Laboratory-scale production of powders and packaging**

The whey permeates (2 lots) were spray dried using a pilot scale spray dryer (Y-105, Shanghai, China) at an inlet air temperature of 180°C and outlet temperature of 83-87°C, feed



temperature less than 20°C, and a pump rate of 6. The RH of the room was periodically recorded using a digital humidity meter (Traceable Humidity Meter, Fisher Scientific, Hampton, NH, USA).

**Table 4.1** Abbreviations used in the study for the spray dried deproteinized whey powders

Samples	Treatment
L1G1	Low lactic acid low galactose
L1G2	Low lactic acid med galactose
L1G3	Low lactic acid high galactose
L2G1	Med lactic acid low galactose
L2G2	Med lactic acid med galactose
L2G3	Med lactic acid high galactose
L3G1	High lactic acid low galactose
L3G2	High lactic acid med galactose
L3G3	High lactic acid high galactose

### Composition

The total solids percentage of the DPW permeates (2 lots) were determined using the oven drying method (AOAC International, 2002; method 990.20). The moisture content of DPW powders was determined in duplicate using the oven method (IDF, 1993). The drying temperature was  $103 \pm 1^\circ\text{C}$ , and the drying time was 4 h. Total nitrogen of DPW powders were analyzed using AOAC (2016) standard methods (990.20). Ash content was determined after the ignition of the sample at  $550^\circ\text{C}$  (AOAC International, 2002; method 954.46). A 5% (w/w) solution of DPW powder was prepared for measuring pH. The pH measurements were done with an Accumet® portable AP63 pH/mV meter (Fisher Scientific, Pittsburgh, PA, USA) calibrated

with standardized buffer solutions (pH 4, 7, and 10) before the measurement. Titratable acidity (% titratable acidity) was done using the method described by Hooi et al. (2004).

### **Water activity**

The water activity ( $a_w$ ) of control and stored DPW powders were determined using water activity meter. Around 1.5 g of DPW powder samples were taken at each data point and were measured by an Aqua Lab CX-2 (Decagon Devices Inc., Pullman, WA, USA).

### **Color**

The  $L^*$ ,  $a^*$ , and  $b^*$  values of control and stored DPW powders were measured using colorimeter (HunterLab, Miniscan XE, Reston, VA).  $L^*$  corresponds to whiteness,  $a^*$  corresponds to green to red on the positive-to-negative scale, and  $b^*$  corresponds to blue to yellow on the positive-to-negative scale. The instrument was standardized using black and white tiles. A plastic petri dish containing the sample (4 mm thickness) was placed above the light source and the  $L^*$ ,  $a^*$ , and  $b^*$  values were recorded. The  $\Delta E$  was determined by the following

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2}$$

where  $L^*$  represents lightness from black (0) to white (100),  $a^*$  represents from green (-) to red (+), and  $b^*$  from blue (-) to yellow (+) for the respective days and  $L_0^*$ ,  $a_0^*$ , and  $b_0^*$  for day 0 (Ferrer et al., 2005).

### **Browning Index**

The browning index (BI) of the powders were determined according to Maskan, (2001). It was determined by measuring the  $L^*$ ,  $a^*$ ,  $b^*$  values using a colorimeter (HunterLab, Miniscan XE, Reston, VA). The BI was determined by the following equation:

$$BI = \frac{100(x - 0.31)}{0.17}$$

where  $x = \frac{a+1.75*L}{5.645*L+a-3.012*b}$

### **FAST Index**

The LS-55 (Perkin Elmer, USA) fluorimeter equipped with a xenon lamp and excitation and emission monochromators were used to obtain the fluorescence emission spectra of tryptophan (Trp) and Maillard products spectra. Trp fluorescence was measured at 290/340 nm. Fluorescence of advanced Maillard products (AMP) was measured at 330/420 nm. Each DPW powder sample was analyzed 3 times. The data was averaged and the peak intensities were used to calculate the FAST index (FI) values. The FI was calculated by the following equation:

$$FI = \frac{F_{AMP}}{F_{TRP}} \times 100$$

### **Hygroscopicity**

The hygroscopicity of DPW powders was measured by the method described by Jaya and Das (2004) with slight modifications. The DPW powder samples (1g) were placed in aluminum pans (Fisher Scientific, USA), which were equilibrated in desiccators with saturated solution with a known relative humidity of sodium chloride (RH=75% at 25°C). Samples were kept at a controlled temperature of 25°C and after 24 h weighed each sample up to the weight difference of no more than 0.5%. Hygroscopicity was measured through the equation:

$$Hy = \frac{((w2 - w1 - w0) \times 1000) + (w1 \times M)}{(w2 - w0) \times 10}$$

where  $w_0$  is the weight (in grams) of the dish,  $w_1$  is the weight (in grams) of the sample,  $w_2$  is the weight (in grams) of the dish after equilibrium has been reached and  $M$  is the moisture content of the sample, in grams per kilogram (Hargrove et al., 1976; Schuck et al., 2012).

## **Caking**

The DPW powder samples were placed at  $102 \pm 2^\circ\text{C}$  for 2 h after the hygroscopicity test and then sieved ( $500 \mu\text{m}$ ) under standard conditions. The caking tendency of a powder can be given by:

$$\text{Caking} = \frac{c}{d} \times 100$$

where  $c$  is the amount of powder left on the sieve after sieving (in grams) and  $d$  is the amount of powder used for sieving (in grams) and the degree of caking is represented in % (Jaya and Das 2004; Schuck et al., 2012).

## **Differential scanning calorimetry**

Thermal properties of DPW powders were determined using a differential scanning calorimeter DSC Q2000 (TA Instruments, DE, USA) equipped with thermal analysis software (TA Instrument Control). The instrument was calibrated using indium. DPW powder (4.5 mg) was directly placed in the aluminum pan, hermetically sealed, and scanned for temperature from 25 to  $220^\circ\text{C}$  at  $5^\circ\text{C}/\text{min}$ . An empty pan was used as a reference. Peak temperature and enthalpy were computed from the thermograms using the data processing software.

## **Microstructure**

The microstructure of DPW powders were examined using a scanning electron microscope. The imaging was conducted using a S-3500N (Hitachi Science Systems Ltd., Tokyo, Japan) and examined by a secondary electron detector operating at 10 kV. The DPW powders were directly mounted onto a carbon double-sided adhesive tape on microscopy stubs and sputter-coated with palladium using a Denton Vacuum Desk II sputter coater (Denton Vacuum, Moorestown, NJ) for 15 min to avoid the charge buildup under the electron beam.

## **Statistical analysis**

Statistical analyses were performed using the procedure PROC GLM of SAS software (SAS version 9.2, SAS Institute Inc., Cary, NC), which involved 2 factors (lactic acid and galactose) each with 3 levels (high, medium, and low). A repeated measures experimental design, with a two-way whole-plot treatment structure, in a completely randomized whole-plot design structure was used. Repeated measurements were taken over time. Lactic acid and galactose, the whole-plot factors, were applied in a  $3 \times 3$  factorial treatment structure. A probability of  $p < 0.05$  was used to establish statistical significance for fixed effects and interactions. All the experimental parameters were measured in duplicates. The data points are reported as mean  $\pm$  standard deviation. All statistical analyses were performed with SAS software.

## **Results and discussion**

### **Composition**

DPW powder composition is summarized in Table 4.2. All samples were within specification for protein, moisture, and ash (American Dairy Products Institute). Table 4.3 shows the probability values from ANOVA of the effects of different lactic acid concentrations, galactose concentrations and storage days on various parameters of deproteinized whey powders.

**Table 4.2** Composition of deproteinized whey powders

Samples*	Total Protein (%)	Moisture (%)	Ash (%)	pH	Titrateable acidity (%)
L1G1	3.41±0.50	1.33±0.03	7.98±0.49	6.68	0.03
L1G2	3.03±0.50	1.59±0.03	8.13±0.53	6.67	0.04
L1G3	3.57±0.25	1.72±0.03	8.05±0.86	6.65	0.04
L2G1	3.06±0.12	1.49±0.23	7.89±0.26	6.23	0.05
L2G2	2.99±0.36	1.50±0.09	7.98±0.35	6.20	0.06
L2G3	2.74±0.14	1.71±0.36	7.88±0.31	6.19	0.06
L3G1	2.63±0.36	1.52±0.30	7.26±0.06	5.88	0.07
L3G2	2.58±0.41	1.66±0.36	7.19±0.16	5.82	0.08
L3G3	2.58±0.14	1.96±0.28	8.09±0.28	5.80	0.08

Results are the means ± standard deviations of data from two independent trials (two lots)

\*Treatments: L1G1 = low lactic acid low galactose; L1G2 = low lactic acid medium galactose; L1G3 = low lactic acid high galactose; L2G1 = medium lactic acid low galactose; L2G2 = medium lactic acid medium galactose; L2G3 = medium lactic acid high galactose; L3G1 = high lactic acid low galactose; L3G2 = high lactic acid medium galactose; L3G3 = high lactic acid high galactose.

**Table 4.3** Probability values from ANOVA of the effects of different lactic acid concentrations, galactose concentrations and storage days on water activity, color (L\*, a\*, b\*), browning index, hygroscopicity, caking and FAST index of deproteinized whey powders.

Source**	NDF	a <sub>w</sub>	L*	a*	b*	BI	FI	Hy	Caking
LA	2	0.1095	0.0207*	0.3603	0.3611	0.1067	0.0033*	0.0068*	0.0100*
GA	2	0.0083*	0.0965	0.1880	0.0483*	0.0070*	0.0001*	0.0036*	0.2573
LA x GA	4	0.0544	0.0192*	0.2990	0.6850	0.1810	0.0001*	0.0382*	0.0072*
Time	6	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.4448
Time x LA	12	0.0092*	0.0522	0.0647	0.5885	0.2758	0.0001*	0.4679	0.5332
Time x GA	12	0.0030*	0.0001*	0.0656	0.1298	0.0223*	0.0001*	0.0614	0.9753
Time x LA x GA	24	0.0362*	0.0001*	0.0001*	0.7563	0.1209	0.0001*	0.8461	0.7613

NDF is the numerator degrees of freedom of the approximate tests, the F tests.

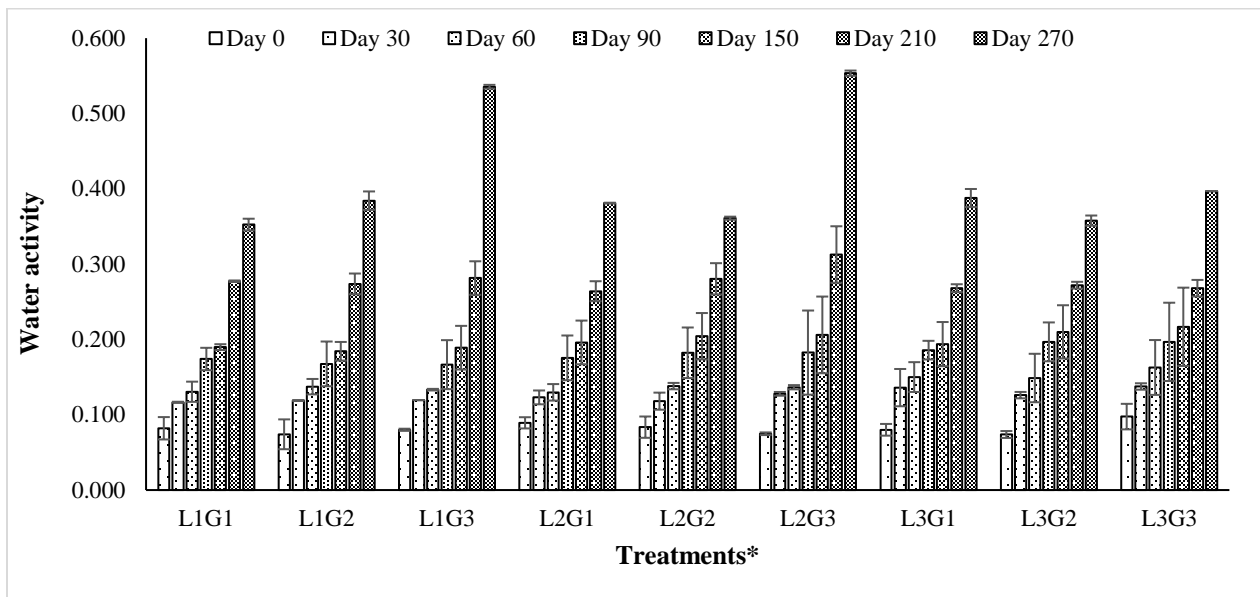
\*The F test was significant at the 0.05 level.

a<sub>w</sub> = water activity, BI = browning index, FI = FAST index, Hy = hygroscopicity

\*\*LA – lactic acid, GA – galactose

## Water activity

Figure 4.1 illustrates the  $a_w$  values of DPW powders during storage at 25°C for 0, 30, 60, 90, 150, 210, and 270 days. The  $a_w$  of DPW powders right after spray drying (fresh powders) were in the range of 0.074 to 0.098. The  $a_w$  of fresh DPW powders for the nine treatments with varying levels of lactic acid and galactose were not significantly ( $p > 0.05$ ) different from each other before storage. In fresh powders, the lowest  $a_w$  of 0.074 was for the sample containing low lactic acid (pH 6.1) medium galactose (0.15%) and high lactic acid (pH 5) medium galactose (0.15%). The highest  $a_w$  of 0.098 was for the sample containing high lactic acid (pH 5) high galactose (0.3%).



**Figure 4.1** Water activity values of deproteinized whey powders during storage at 25°C for 0, 30, 60, 90, 150, 210, and 270 days.

Error bars are standard deviations from two independent trials (two lots).

\*Treatments: L1G1 = low lactic acid low galactose; L1G2 = low lactic acid medium galactose; L1G3 = low lactic acid high galactose; L2G1 = medium lactic acid low galactose; L2G2 = medium lactic acid medium galactose; L2G3 = medium lactic acid high galactose; L3G1 = high lactic acid low galactose; L3G2 = high lactic acid medium galactose; L3G3 = high lactic acid high galactose.

It was observed that the  $a_w$  of DPW powders increased gradually with increasing storage time. The  $a_w$  values were not significantly ( $p > 0.05$ ) different from storage days 0 through 60. However, the  $a_w$  values increased significantly ( $p < 0.05$ ) on the storage of DPW powders for 210 and 270 days from all the other storage days.

In DPW powders, the main effect galactose was found to have a significant ( $p < 0.05$ ) effect on  $a_w$  values. On storage of DPW powders, time and the interaction effects time x lactic acid, time x galactose, time x lactic acid x galactose each were also found to have a significant ( $p < 0.05$ ) effect on  $a_w$  values (Table 4.3). On 270 days of storage, the highest  $a_w$  was found to be for the samples containing medium lactic acid (pH 5.55) high galactose (0.3%) followed by low lactic acid (pH 6.1) high galactose (0.3%) followed by high lactic acid (pH 5) high galactose (0.3%). This could be due to the presence of galactose in these samples. Galactose like many other monosaccharides is hygroscopic in nature. Torres et al. (2017) observed that it was more difficult to dry lactose hydrolyzed milk powder when compared to whole milk powder because of the hygroscopicity of its constituents.

The introduction of lactic acid and galactose can make the DPW powders more hygroscopic in nature. Lactic acid is a highly hygroscopic compound with a  $T_g$  value of approximately  $-60^\circ\text{C}$  (Maltini et al., 1997) whereas galactose has a  $T_g$  of  $30^\circ\text{C}$  (Roos, 2002). Chandrapala and Vasiljevic (2017) reported an increase in  $a_w$  with increasing lactic acid content in spray dried lactose powders from 0.328 (lactose powder) to 0.392 (lactose + 1% lactic acid powder). In DPW powders, the  $a_w$  was 0.082 for the sample containing low lactic acid (pH 6.1) low galactose (0%) (control) and 0.080 for sample containing high lactic acid (pH 5) low galactose (0%) before storage. On 270 days of storage, the  $a_w$  increased from 0.353 for the control to 0.388 for the sample containing high lactic acid (pH 5) low galactose (0%), however, a



$a_w$  value of 0.536 was observed for the sample containing low lactic acid (pH 6.1) high galactose (0.3%). In comparison to 1% lactic acid in their study, our study had 0.4% lactic acid for the high lactic acid treated samples. In contrast to many studies where the presence of lactic acid causes the powders to absorb moisture, interestingly this study demonstrated that the  $a_w$  increased for high galactose samples for DPW powders during storage. The  $a_w$  has an influence on other chemical and physical properties of DPW powders.

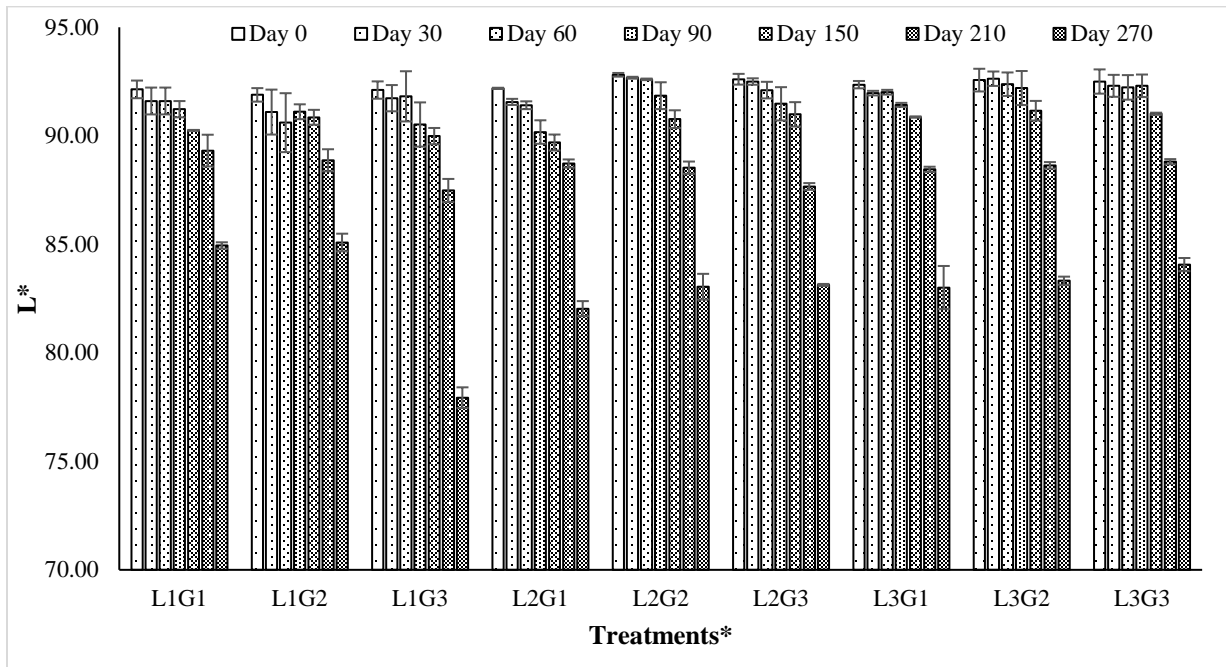
### **Color**

The  $L^*$ ,  $a^*$  (data not shown), and  $b^*$  values of fresh DPW powders right after spray drying were found to be in the range 91.89 to 92.81, -0.95 to -0.63 and 12.33 to 14.71, respectively. The nine treatments with varying levels of lactic acid and galactose were not found to be significantly ( $p > 0.05$ ) different for DPW powders right after production, indicative of uniform drying in the spray dryer for all the treatment combinations. All the powders were dried under the same conditions with an inlet temperature of 180°C and outlet temperature maintained at 82-87°C, respectively. The moisture content of the DPW powders right after spray drying ranged from 1.33 to 1.96%, although, it was not found to be significantly ( $p > 0.05$ ) different.  $L^*$ ,  $b^*$ , and  $\Delta E$  are considered important variables to explain Maillard browning (Banavara et al., 2003). The main effects lactic acid and galactose nor the interaction effect lactic acid x galactose did not have any significant ( $p > 0.05$ ) effect on  $a^*$  values and thus were not discussed in detail.

**$L^*$ .** The  $L^*$  values of the DPW powders during storage at 25°C for 0, 30, 60, 90, 150, 210 and 270 days is shown in Figure 4.2. In general, it was observed that the  $L^*$  values did not show any significant ( $p > 0.05$ ) change until 150 days of storage, however, it showed significant ( $p < 0.05$ ) decrease on storage days 210 and 270. The  $L^*$  values of the control (low lactic acid (pH 6.1) low galactose (0%)) showed a decrease from 92.14 to 90.25 for 0 and 150 days of storage,

respectively, after which it decreased significantly to 84.95 by 270 days of storage. In DPW powders during storage, the main effects lactic acid had a significant effect, however, galactose did not have a significant ( $p > 0.05$ ) effect on  $L^*$  values. The interaction term lactic acid x galactose was found to have a significant ( $p < 0.05$ ) effect. On storage, time and the interaction effects time x galactose, time x lactic acid x galactose each were also found to have a significant ( $p < 0.05$ ) effect on the  $L^*$  values (Table 4.3). The high galactose samples exhibited greater degree of browning and showed decreasing  $L^*$  values in the order high lactic acid (pH 5) high galactose (0.3%) > medium lactic acid (pH 5.55) high galactose (0.3%) > low lactic acid (pH 6.1) high galactose (0.3%). This could be due to the presence of galactose. These results were in agreement with Dattatreya et al. (2010) who reported that samples which contain galactose (1.5 g/L) have a greater degree of browning when compared to those containing just lactose (3.0 g/L) indicating that residual sugars have a significant effect on the browning in sweet whey powders. In our study, the galactose content used was 0.15% (0.675g/L) and 0.30% (1.35 g/L) compared to 1.5 g/L used in their study. The rationale for this being that the presence of sugars such as galactose could increase the initial rate of reducing sugar- dependent browning when it reacts with a given amino compound primarily by increasing the rate at which the sugar's ring opens to a reactive, reducible form (Overend et al., 1961; Dattatreya et al., 2010). Interestingly, the sample containing high lactic acid (pH 5) high galactose (0.3%) had  $L^*$  value higher when compared to the sample containing low lactic acid (pH 6.1) high galactose (0.3%) and medium lactic acid (pH 5.55) high galactose (0.3%) during storage. This could be due to the fact that acidic conditions impede browning. Similar results were observed by Buera et al. (1990) where lowering the initial pH decreased the rate of color development in hydrolyzed concentrated (50%) cheese whey model system for the pH range 4.5-6. Dattatreya et al. (2010) reported that

for the storage of galactose lysine model system at 80°C for 24 hours, the L\* values increased as pH decreased. However, the reverse was true for galactose treated samples in sweet whey powder model system. This could be due to the presence of minerals and salts in the sweet whey powder system which accelerates the Maillard reactions (Burin et al., 2000).



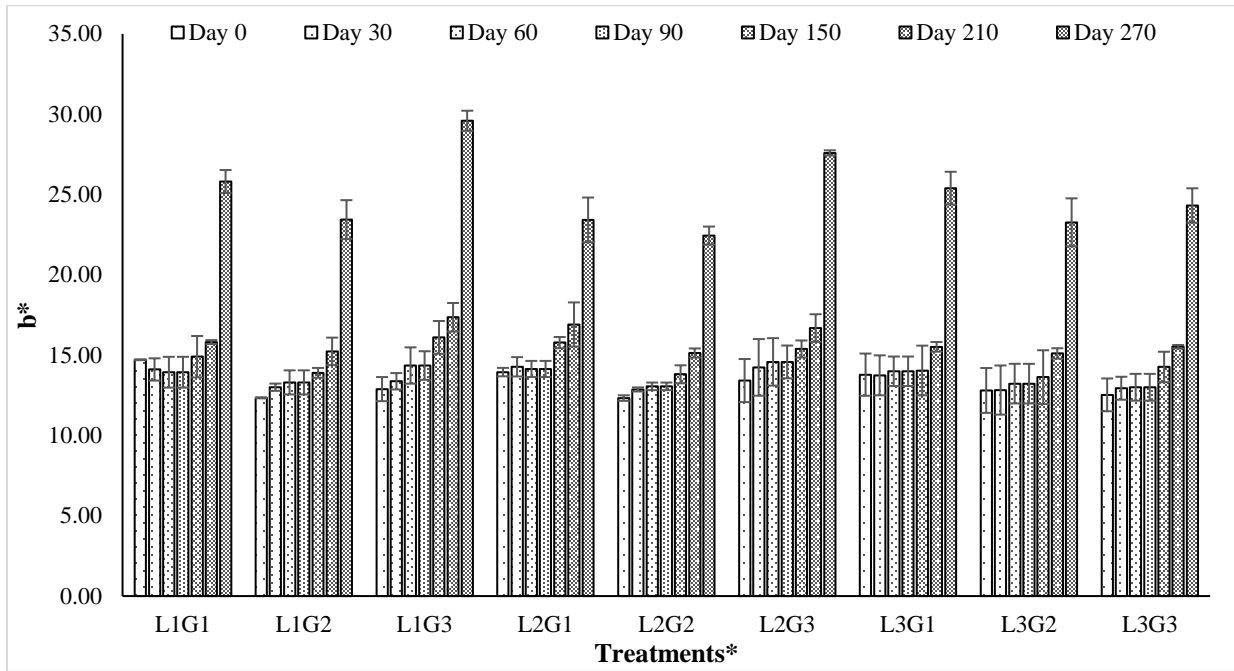
**Figure 4.2** L\* values of deproteinized whey powders during storage at 25°C for 0, 30, 60, 90, 150, 210, and 270 days.

Error bars are standard deviations from two independent trials (two lots).

\*Treatments: L1G1 = low lactic acid low galactose; L1G2 = low lactic acid medium galactose; L1G3 = low lactic acid high galactose; L2G1 = medium lactic acid low galactose; L2G2 = medium lactic acid medium galactose; L2G3 = medium lactic acid high galactose; L3G1 = high lactic acid low galactose; L3G2 = high lactic acid medium galactose; L3G3 = high lactic acid high galactose.

**b\***. The b\* values of the DPW powders during storage at 25°C for 0, 30, 60, 90, 150, 210, and 270 days is shown in Figure 4.3. It was observed that the b\* values did not show any significant ( $p > 0.05$ ) change until 210 days of storage after which it increased significantly ( $p < 0.05$ ) on 270 days of storage. The b\* values for DPW powders on day 0 (before storage) and day 210 were in the range 12.33 to 14.71 and 15.11 to 17.36, respectively. In contrast, the b\* values showed a sudden increase by 270 days of storage which were in the range 22.45 to 29.60. The

increase in  $b^*$  values could be attributed to the higher concentration of yellow-colored compounds formed during the Maillard reaction.



**Figure 4.3**  $b^*$  values of deproteinized whey powders during storage at 25°C for 0, 30, 60, 90, 150, 210 and 270 days.

Error bars are standard deviations from two independent trials (two lots).

\*Treatments: L1G1 = low lactic acid low galactose; L1G2 = low lactic acid medium galactose; L1G3 = low lactic acid high galactose; L2G1 = medium lactic acid low galactose; L2G2 = medium lactic acid medium galactose; L2G3 = medium lactic acid high galactose; L3G1 = high lactic acid low galactose; L3G2 = high lactic acid medium galactose; L3G3 = high lactic acid high galactose.

The  $b^*$  values for the DPW powders during storage were significantly affected by galactose and time (Table 4.3). It was observed that the samples containing low lactic acid (pH 6.1) high galactose (0.3%) followed by medium lactic acid (pH 5.55) high galactose (0.3%) had the most color change at 270 days of storage which could be attributed to the high galactose content present in these powders. Remarkably, the sample containing high lactic acid (pH 5) high galactose (0.3%) had a  $b^*$  value lesser when compared to the other high galactose treated samples (medium lactic acid (pH 5.55) high galactose (0.3%) and low lactic acid (pH 6.1) high

galactose (0.3%). This could be attributed to the galactose content present and its higher reaction rate with lysine when compared to lactose (Naranjo et al., 2013).

**ΔE.** It was observed that the ΔE values increased with increasing storage time. Table 4.4 represents the ΔE values of DPW powders on 30, 60, 90, 150, 210, and 270 days of storage.

**Table 4.4** The mean color change (ΔE) values of deproteinized whey powders on 30, 60, 90, 150, 210, and 270 days of storage.

Samples	Day 30	Day 60	Day 90	Day 150	Day 210	Day 270
L1G1	0.84	1.01	1.02	1.94	3.03	13.38
L1G2	1.29	1.63	1.78	1.91	4.19	13.09
L1G3	0.62	1.50	3.59	3.86	6.44	22.46
L2G1	0.75	0.81	2.73	3.11	4.56	14.15
L2G2	0.49	0.79	1.79	2.59	5.14	14.31
L2G3	0.84	1.28	2.28	2.56	5.94	17.17
L3G1	0.44	0.55	1.04	1.57	4.28	15.15
L3G2	0.08	0.50	0.91	1.64	4.55	14.07
L3G3	0.41	0.61	1.77	2.34	4.77	14.56

\*Treatments: L1G1 = low lactic acid low galactose; L1G2 = low lactic acid medium galactose; L1G3 = low lactic acid high galactose; L2G1 = medium lactic acid low galactose; L2G2 = medium lactic acid medium galactose; L2G3 = medium lactic acid high galactose; L3G1 = high lactic acid low galactose; L3G2 = high lactic acid medium galactose; L3G3 = high lactic acid high galactose.

The development of brown color is a characteristic of powders with storage due to Maillard browning. In DPW powders, there is an increased tendency of Maillard browning due to the high content of lactose present (Labuza and Saltmarch, 1982). The ΔE values ranged from 0.08 to 0.84 (day 30), 1.57 to 3.86 (day 150), and 3.03 to 5.94 (day 210). On the other hand, day 270 was notably different ranging from 13.09 to 22.64. In this research, the ΔE values were found to increase with storage. Similar results were observed by Dattatreya and Rankin (2006) who observed an increase in ΔE values with time during accelerated storage (80°C) of sweet whey powder. On 270 days of storage, the highest ΔE values of 22.64 and 17.17 were for the

samples containing low lactic acid (pH 6.1) high galactose (0.3%) and medium lactic acid (pH 5.55) high galactose (0.3%), respectively. This could be due to the galactose content present in these samples.  $\Delta E$  is the most sensitive indicator of the effects of both time and treatment on the progress of the Maillard reaction. Le et al. (2011) also noted that  $\Delta E$  was the most sensitive indicator of  $a_w$  on the progress of the Maillard reaction.

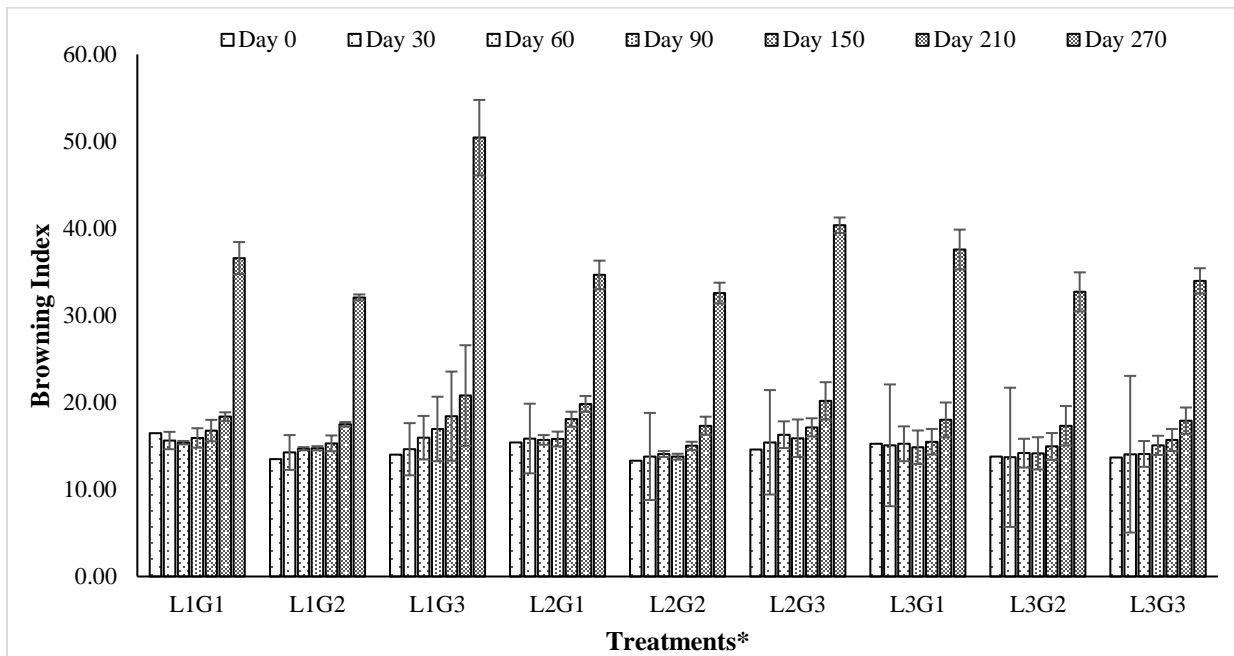
The DPW powders had a decrease in lightness and increase in redness and yellowness during storage at 25°C, however being stored at a lower temperature, the changes were only minimal until 150 or 210 days of storage after which it had a significant increase. It should be considered that in a multicomponent system like DPW the Maillard reactions occurring may be complicated due to the range of different compounds that act as reactants.

### **Browning index**

Browning is an indicator of powder acceptability in terms of quality which limits shelf life (Saltmarch and Labuza, 1982; Sithole et al., 2005). The BI values of DPW powders during storage at 25°C for 0, 30, 60, 90, 150, 210, and 270 days is given in Figure 4.4. The BI of DPW powders right after production was found to be in the range 13.32 to 16.47 (AU). It was observed that the DPW powders with varying levels of lactic acid and galactose did not show any significant ( $p > 0.05$ ) difference for BI values before storage.

It was observed that the BI values did not have a significant ( $p > 0.05$ ) change until 210 days of storage. On the other hand, the BI values showed a noticeable increase on 270 days of storage which was significantly ( $p < 0.05$ ) different. The BI values on 270 days of storage were in the range of 32.07 to 50.43. The BI values increased during storage possibly due to the Maillard browning in DPW powders caused by the reaction between lactose and protein. These results were in agreement with Burin et al. (2000) who observed that the dairy powders brown

with the increase in storage time, but in their study, the BI measurements were obtained by reconstituting the powder in water. These authors also observed that sweet whey powder had the highest browning rate when compared to reduced mineral whey, whey protein concentrate, and whey protein isolate at a given storage temperature. Schebor et al. (1999) reported an increase in non-enzymatic browning with increasing temperatures (45 and 60°C) and with increasing storage time (0-200 days) in skim milk powders using BI values.



**Figure 4.4** Browning index of deproteinized whey powders during storage at 25°C for 0, 30, 60, 90, 150, 210, and 270 days.

Error bars are standard deviations from two independent trials (two lots).

\*Treatments: L1G1 = low lactic acid low galactose; L1G2 = low lactic acid medium galactose; L1G3 = low lactic acid high galactose; L2G1 = medium lactic acid low galactose; L2G2 = medium lactic acid medium galactose; L2G3 = medium lactic acid high galactose; L3G1 = high lactic acid low galactose; L3G2 = high lactic acid medium galactose; L3G3 = high lactic acid high galactose.

In DPW powders, it was observed that the main effect galactose had a significant ( $p < 0.05$ ) effect on BI values. On storage, the BI values were significantly affected by time and the interaction term time x galactose (Table 4.3). The highest BI on 270 days of storage was found to be for the sample containing low lactic acid (pH 6.1) high galactose (0.3%) followed by medium

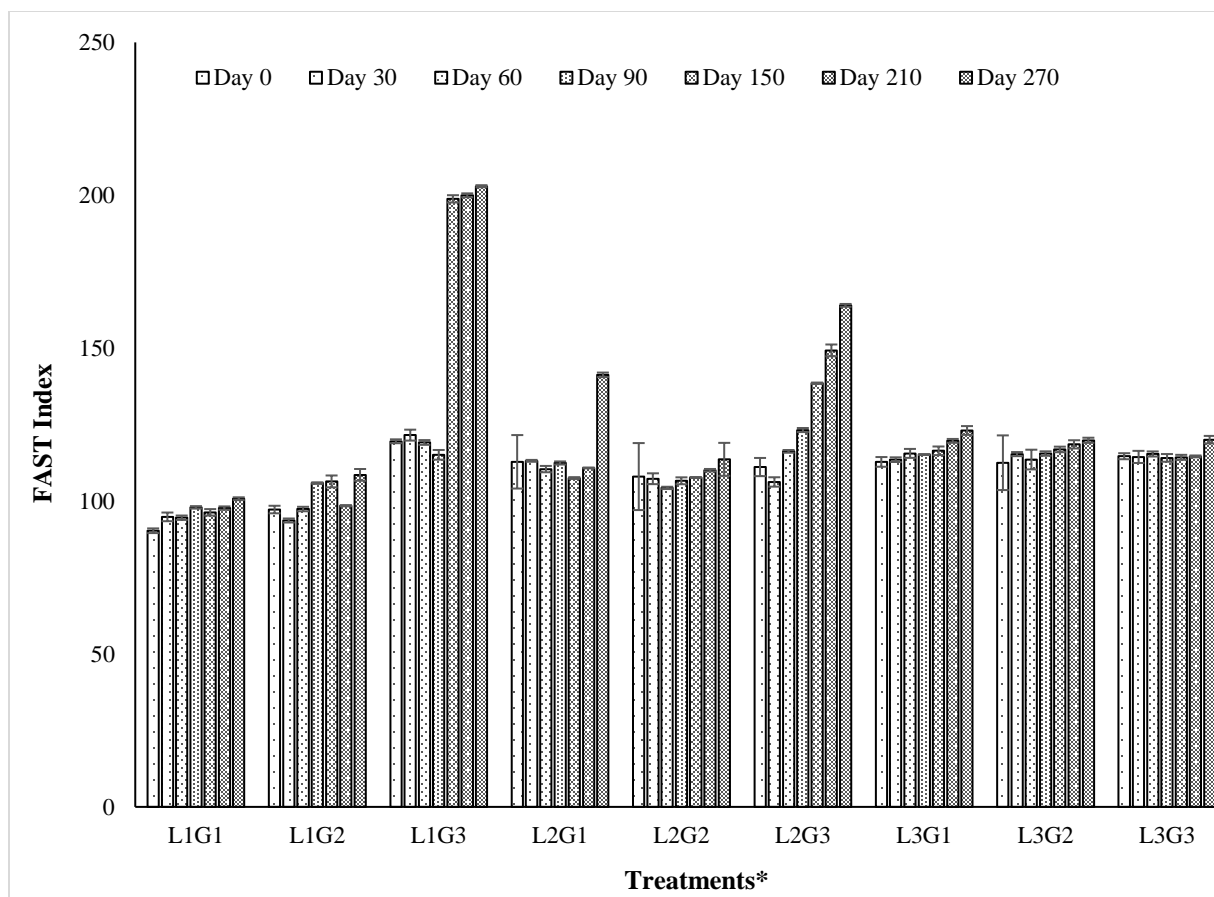
lactic acid (pH 5.55) high galactose (0.3%) with values 50.43 and 40.36 (AU), respectively. However, for the sample containing high lactic acid (pH 5) high galactose (0.3%), the BI value was observed to be 33.97 (AU) which was low when compared to the other high galactose treated samples. The BI values of high lactic acid samples ranged from 32.70 to 37.57 (AU) on 270 days of storage.

The sudden increase in BI values from 210 to 270 days could be linked to the increase in  $a_w$  of the DPW powders. Also, it was observed that the samples with the highest  $a_w$  had the highest browning which indicates that  $a_w$  and browning of DPW powders are interrelated. Labuza and Saltmarch (1981) reported that maximum browning happens at the  $a_w$  range 0.50 to 0.75. Also, with the increase in  $a_w$  from 0.33 to 0.54 the browning rate increases and then plateaus above that (Bell, 1995). At lower  $a_w$  values, the browning reactions tend to proceed slowly which could be due to limited reactant mobility which resulted in less browned powders (Eichner and Karel, 1972; Labuza, 1970).

### **FAST Index**

The FI has been reported to be an effective method to follow the Maillard reaction as fluorescent products will be formed as indicators of the Maillard reaction (Birlouez-Aragon et al., 2001; Matiacevich and Buera, 2006). The FI values of DPW powders during storage at 25°C for 0, 30, 60, 90, 150, 210, and 270 days is as shown in Figure 4.5. The FI of fresh DPW powders ranged from 90 to 119. It was found that the fresh DPW powders right after production with varying levels of lactic acid and galactose were significantly ( $p < 0.05$ ) different only for the sample containing low lactic acid (pH 6.1) high galactose (0.3 %) which had the highest FI value of 119 among fresh powders.





**Figure 4.5** FAST index of deproteinized whey powders during storage at 25°C for 0, 30, 60, 90, 150, 210, and 270 days.

Error bars are standard deviations from two independent trials (two lots).

\*Treatments: L1G1 = low lactic acid low galactose; L1G2 = low lactic acid medium galactose; L1G3 = low lactic acid high galactose; L2G1 = medium lactic acid low galactose; L2G2 = medium lactic acid medium galactose; L2G3 = medium lactic acid high galactose; L3G1 = high lactic acid low galactose; L3G2 = high lactic acid medium galactose; L3G3 = high lactic acid high galactose.

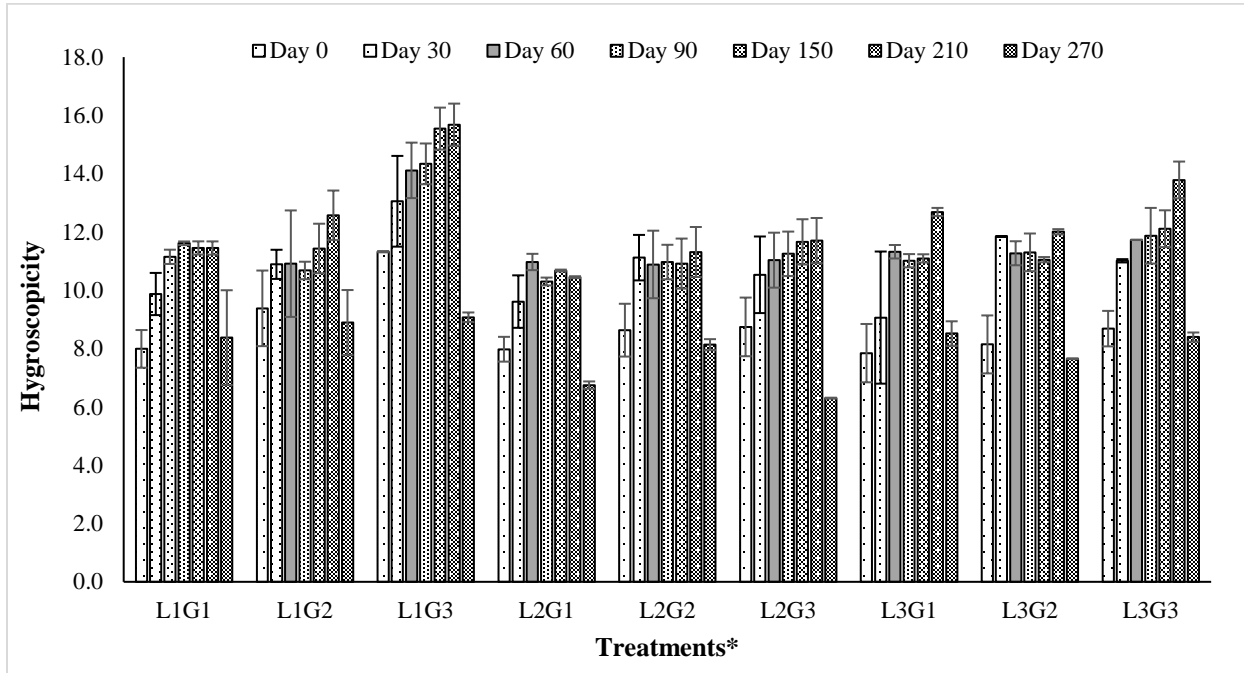
The FI values did not change significantly ( $p > 0.05$ ) until 60 days of storage. Also, the FI values were not significantly different for 150 and 210 days of storage. However, it showed a significant ( $p < 0.05$ ) difference on 270 days of storage from all the other storage days. The increase in FI values could be indicative of the Maillard reaction occurring in the DPW powders during storage. According to Table 4.3, the FI values were significantly ( $p < 0.05$ ) affected by the main effects lactic acid, galactose, and the interaction term lactic acid x galactose. The FI

values were also significantly ( $p < 0.05$ ) affected by time and the interaction terms time x lactic acid, time x galactose, and time x lactic acid x galactose during storage. On 270 days of storage, the FI values were found to be the lowest for the sample containing low lactic acid (pH 6.1) low galactose (0 %) (control). The highest FI values were for the samples containing low lactic acid (pH 6.1) high galactose (0.3%) followed by the sample containing low lactic acid (pH 6.1) medium galactose (0.15%) with values 202 and 164, respectively. Desic and Birlouez-Aragon (2010) used FI to study the heat impact of the infant formula model and suggested FI as a good alternative for Maillard reaction monitoring in milk products. The FAST method was shown to correlate with several Maillard indicators including furosine and carboxymethyllysine (Liogier et al., 2014). The lesser the storage time, the lower the changes in the molecular environment and consequently, the higher is the Trp fluorescence with mild AMP accumulation resulting in low FI values. On the other hand, during storage, the Trp decreases and significant AMP accumulation results in higher FI. In conclusion, all the heating steps, including concentration and spray drying, are associated with different rates of Maillard products formation. Additionally, storage time influences the rate of Maillard products formed.

### **Hygroscopicity**

Hygroscopicity of a powder can be defined as the ability of a powder to uptake moisture after exposure to a high humidity environment under normal conditions. According to Pisecky powder classification, a powder with hygroscopicity values  $\leq 10\%$  is considered a non-hygroscopic powder and the one with hygroscopicity values  $\geq 25\%$  is considered an extremely hygroscopic powder (Pisecky, 1997; Schuck et al., 2004). The hygroscopicity of DPW powders on 0, 30, 60, 90, 150, 210, and 270 days of storage at 25°C is shown in Figure 4.6. The hygroscopicity of DPW powders before storage ranged from 7.8 to 11.3% which were

significantly ( $p < 0.05$ ) different. Previously, Sithole et al. (2006) reported hygroscopicity values of commercial sweet whey powder collected from various geographical locations to be between 14.7 to 25.1% which were higher than the values observed in our study.



**Figure 4.6** Hygroscopicity values of deproteinized whey powders during storage at 25°C for 0, 30, 60, 90, 150, 210, and 270 days.

Error bars are standard deviations from two independent trials (two lots).

\*Treatments: L1G1 = low lactic acid low galactose; L1G2 = low lactic acid medium galactose; L1G3 = low lactic acid high galactose; L2G1 = medium lactic acid low galactose; L2G2 = medium lactic acid medium galactose; L2G3 = medium lactic acid high galactose; L3G1 = high lactic acid low galactose; L3G2 = high lactic acid medium galactose; L3G3 = high lactic acid high galactose.

The hygroscopicity values were not significantly ( $p > 0.05$ ) different until 30 days of storage. Also, it was not significantly ( $p > 0.05$ ) different from 30 days until 210 days of storage. The hygroscopicity values did not show any significant ( $p > 0.05$ ) difference between 0 and 270 days of storage which could be due to the sudden decrease from 210 to 270 days of storage. The main effects lactic acid, galactose, and interaction term lactic acid x galactose were found to have a significant ( $p < 0.05$ ) effect on hygroscopicity values. The hygroscopicity values were

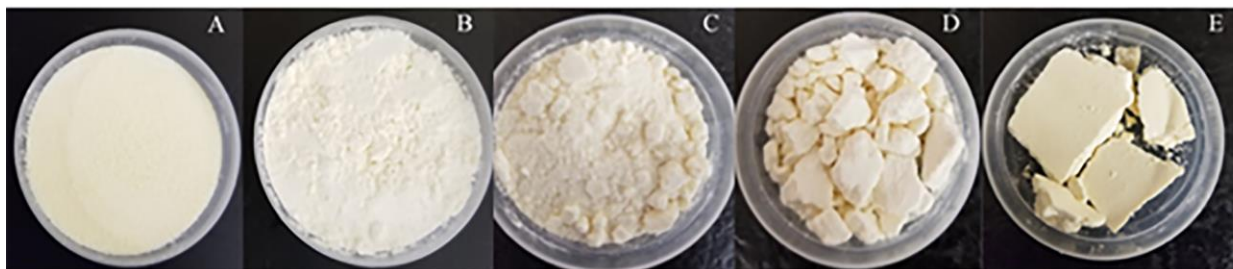
also found to have a significant ( $p < 0.05$ ) effect with time during storage (Table 4.3). The galactose has a low  $T_g$  of  $30^\circ\text{C}$  which can cause an increased tendency for stickiness and agglomeration in powders (Rao et al., 2004). Hargrove et al. (1976) reported that there was a direct relationship between hygroscopicity and the amount of lactic acid present in the samples. This could lead to increased hygroscopicity of DPW powders with samples containing lactic acid and galactose.

The hygroscopicity values for DPW powders on 210 and 270 days of storage were in the range 10.5 to 15.7 and 6.3 to 9.1, respectively. This sudden dip in hygroscopicity values from 210 to 270 days of storage could be because the DPW powders could no longer take in any more moisture as it was already caked thus not giving a predicted result of increased values. The DPW powders changed from a free-flowing non-hygroscopic powder to hygroscopic powder by the storage period of 210 days that has an increased risk of absorbing moisture from the environment which induces caking according to Pisecky powder classification (Pisecky, 1997; Schuck et al., 2004). The hygroscopicity values of 270 days of storage does not give a clear idea if the powder became extremely hygroscopic or not due to the sudden decrease in the values, however, by visual observation, it was evident that the DPW powders on 270 days of storage had caked completely.

## **Caking**

Caking is a detrimental phenomenon in food powders. Caking of powders can be defined as an undesirable change in which the powders tend to stick together to form lumps (Aguilera et al., 1995). Researchers consider caking as an advanced stage to sticky powder which includes the formation of solid bridges (Listiohadi et al., 2005; Kelly, 2009). Huppertz and Gazi (2016) reported that lactose is known to have an intense effect on the behavior and properties of

ingredients with a high content (more than 50% DM) of lactose during processing and storage. The degree of caking of fresh DPW powders were found to be in the range 97.54 to 98.59%. The DPW powder just after spraying was a free-flowing powder however it was observed that the caking value of DPW powders were high for the fresh powders itself which could be due to the increased tendency of these powders to absorb moisture due to the high lactose content present. Figure 4.7 shows the images of DPW powder sample containing medium lactic acid (pH 5.55) high galactose (0.3%) showing caking on 0, 30, 150, 210, and 270 days of storage. The degree of caking of DPW powders on 270 days of storage ranged from 97.88 to 98.70%. The increase in the degree of caking with storage time was evident from visual observations as shown in Figure 4.7 however it is not understood by the caking values. This could be because the method used for determination of the degree of caking was not an effective method to follow the caking of DPW powders. Schuck et al. (2012) reported that this method was unstandardized given the limitations in understanding the caking phenomenon taking into consideration the composition and physical properties of the powder. It was observed that the sample containing medium lactic acid (pH 5.55) high galactose (0.3%) caked when compared to other treatments which is shown in Figure 4.7. It was followed by low lactic acid (pH 6.1) high galactose (0.3%) by visual examination.



**Figure 4.7** Images of deproteinized whey powder (medium lactic acid (pH 5.55) high galactose (0.3%)) showing caking on different days of storage (A) Day 0 (B) Day 30 (C) Day 150 (D) Day 210 and (E) Day 270 at 25°C.

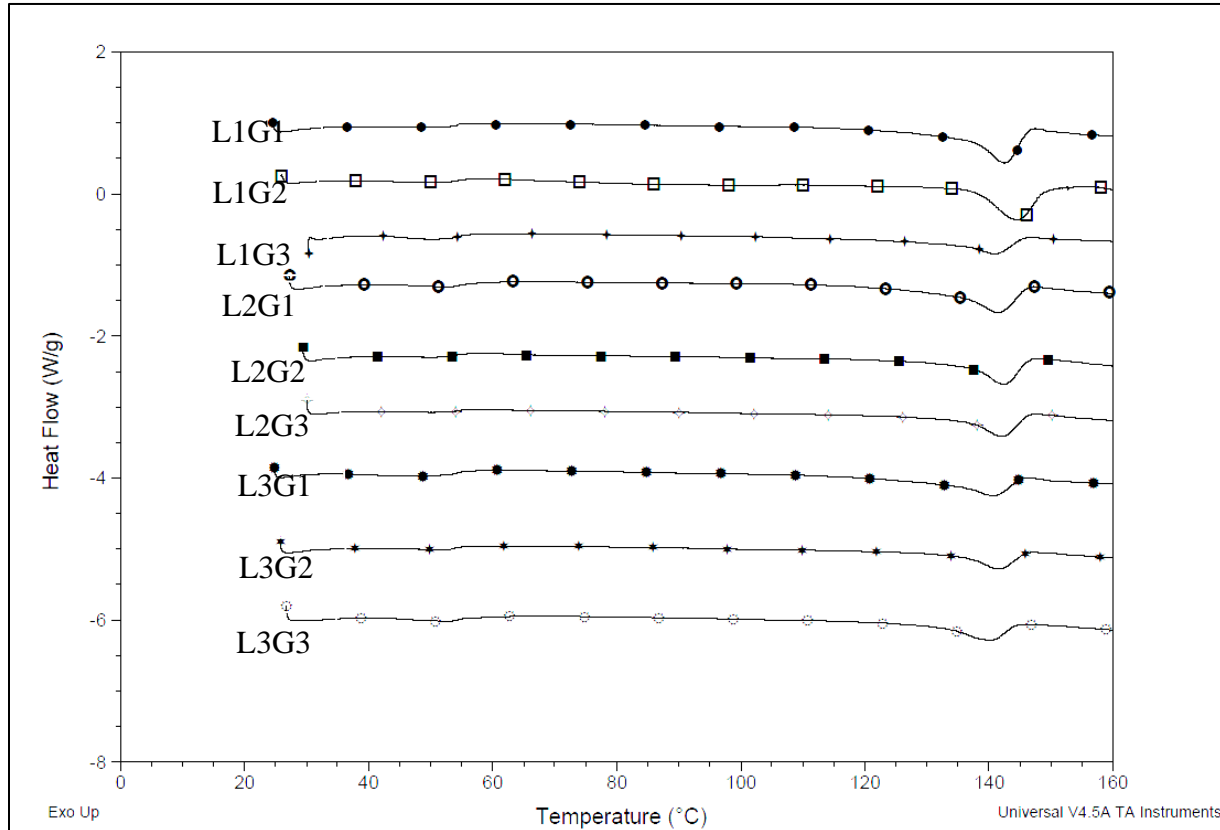
The DPW powders right after production (day 0) were non-hygroscopic free-flowing powders but after 30 days of storage, the powders were seen as slightly bigger particles than the

fresh powder, with particles coming together due to cohesion. The cohesion or stickiness between particles is largely affected by the surface structure (Chen and Özkan, 2007). The powder started forming small agglomerated particles by 150 days of storage. Hartmann and Palzer, (2011) reported that the powder particles adhere to each other and start to get sticky often referred to as stickiness, that decreases the flowability of the powder in the primary stages of the caking process. This occurred as lactose crystals in the powders started absorbing moisture. However, by day 210 the powders started caking and can be seen as agglomerated caked particles or as lumps which can be broken if force is applied. The reason for this being that the crystalline lactose starts to partially dissolve as the critical humidity is exceeded. This forms liquid bridges between the lactose particles which are friable and hence, are easier to break when formed. The strength of liquid bridges can be dependent on the amount of water adsorbed (Teunou and Fitzpatrick, 1999). The DPW powders completely caked as a harder lump which was hard to break by 270 days of storage. The liquid bridges formed between the particles are high in dissolved lactose and after a point, it gets saturated, which causes the lactose to recrystallize with supersaturation being the driving force. When this happens and the water is evaporated out of this, it forms solid bridges between the particles thus making it an unbreakable lump.

### **Differential scanning calorimetry**

The DSC thermograms of DPW powders right after production is shown in Figure 4.8. It can be observed clearly that there is an endothermic peak at  $\sim 142^{\circ}\text{C}$  for the DPW powders which represent the loss of crystalline water. Similar results were observed by Gombas et al. (2002) for crystalline lactose which showed an endothermic peak at  $144^{\circ}\text{C}$ . This endothermic peak shows a peak shift for the treatments which could be due to the addition of lactic acid and galactose to the

samples. It has been reported that although lactose has a  $T_g$  of  $101^\circ\text{C}$ , its monosaccharide units glucose and galactose have a much lower  $T_g$  of  $31^\circ\text{C}$  and  $30^\circ\text{C}$ , respectively (Roos, 1993). Lactic acid is very hygroscopic and has a very low  $T_g$  of  $-60^\circ\text{C}$  (Maltini et al., 1997; Chandrapala and Vasiljevic, 2017). The rate of nonenzymatic browning is extremely low below the  $T_g$ , whereas, at temperatures greater than the  $T_g$ , this rate is higher (Karmas et al., 1992).

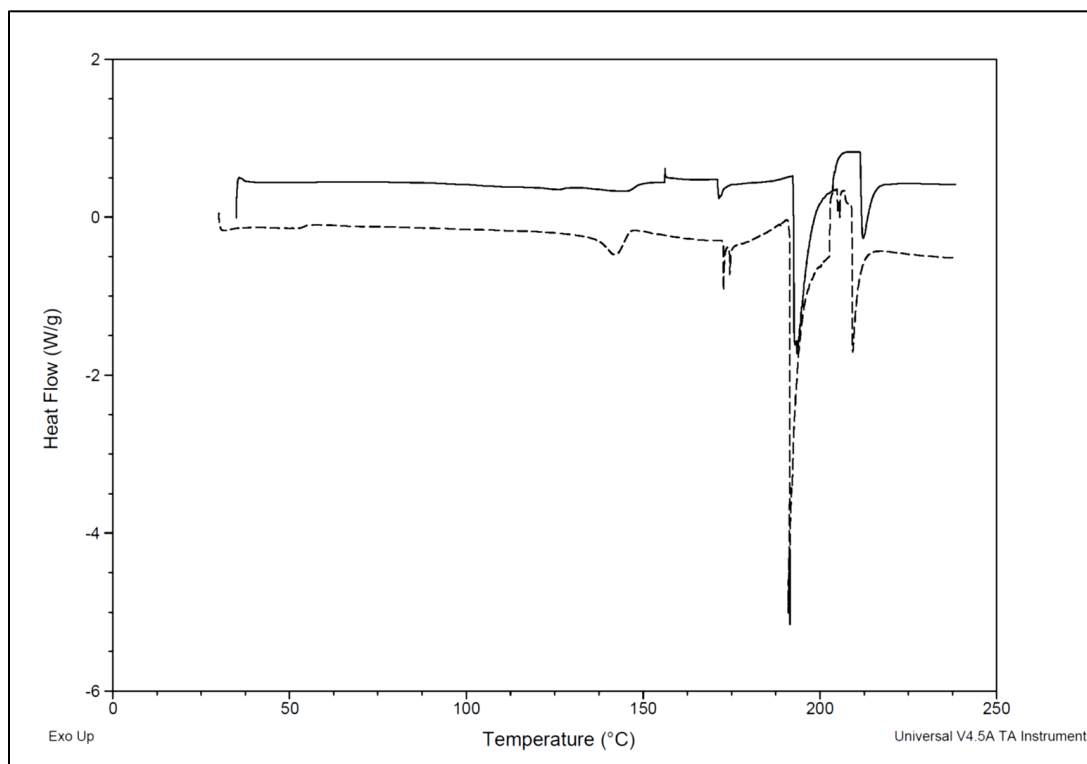


**Figure 4.8** Thermograms of deproteinized whey powders before storage.

\*Treatments: L1G1 = low lactic acid low galactose; L1G2 = low lactic acid medium galactose; L1G3 = low lactic acid high galactose; L2G1 = medium lactic acid low galactose; L2G2 = medium lactic acid medium galactose; L2G3 = medium lactic acid high galactose; L3G1 = high lactic acid low galactose; L3G2 = high lactic acid medium galactose; L3G3 = high lactic acid high galactose.

Thermographic analyses of DPW sample containing medium lactic acid (pH 5.55) high galactose (0.3%) during storage from 0 to 270 day is shown in Figure 4.9. The obtained thermograms showed an endothermic peak around  $143^\circ\text{C}$ , which can be attributed to the removal

of crystalline water (Wijayasinghe et al., 2015; Yazdanpanah and Langrish, 2011). The peak enthalpy before storage was 19.50 J/g, although, after storage, the enthalpy decreased to ~ 6.98 J/g for the sample medium lactic acid (pH 5.55) high galactose (0.3%). The amount of water removed was reduced by 65% when stored for 270 days. There were two endothermic peaks observed at 192°C and 212°C, respectively corresponding to  $\alpha$ -melt. Gombas et al. (2002) reported an endothermic peak at 213°C corresponding to  $\alpha$ -melt for 100% crystalline  $\alpha$ -lactose monohydrate. Chandrapala et al. (2016) also observed  $\alpha$ -melt at 218°C for pure lactose samples. However, the authors also reported that the presence of lactic acid led to the appearance of an additional endothermic peak in lactose + lactic acid model systems.

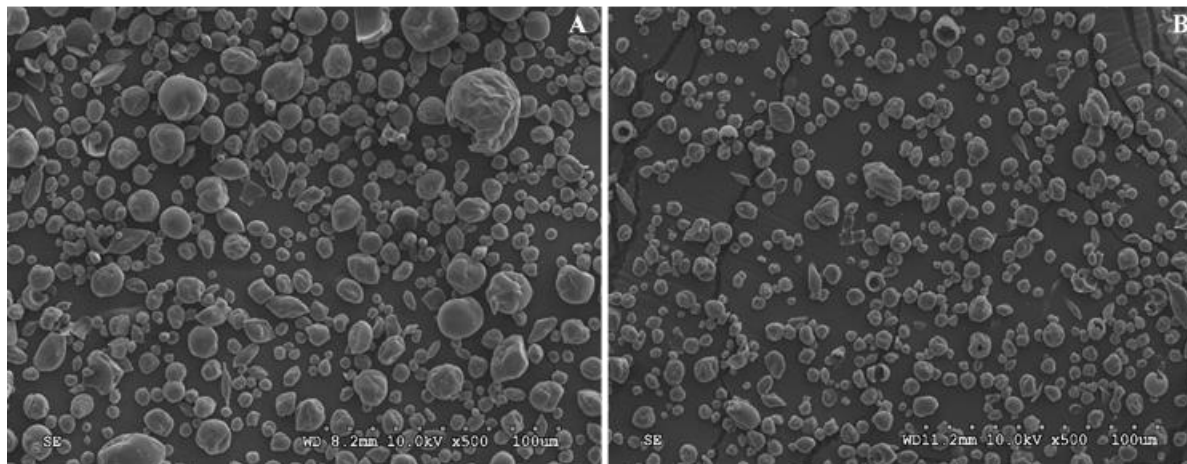


**Figure 4.9** Thermograms of deproteinized whey powder containing medium lactic acid (pH 5.55) high galactose (0.3%) before (0 day; dotted line) and after storage (270 day; solid line).



## Microstructure

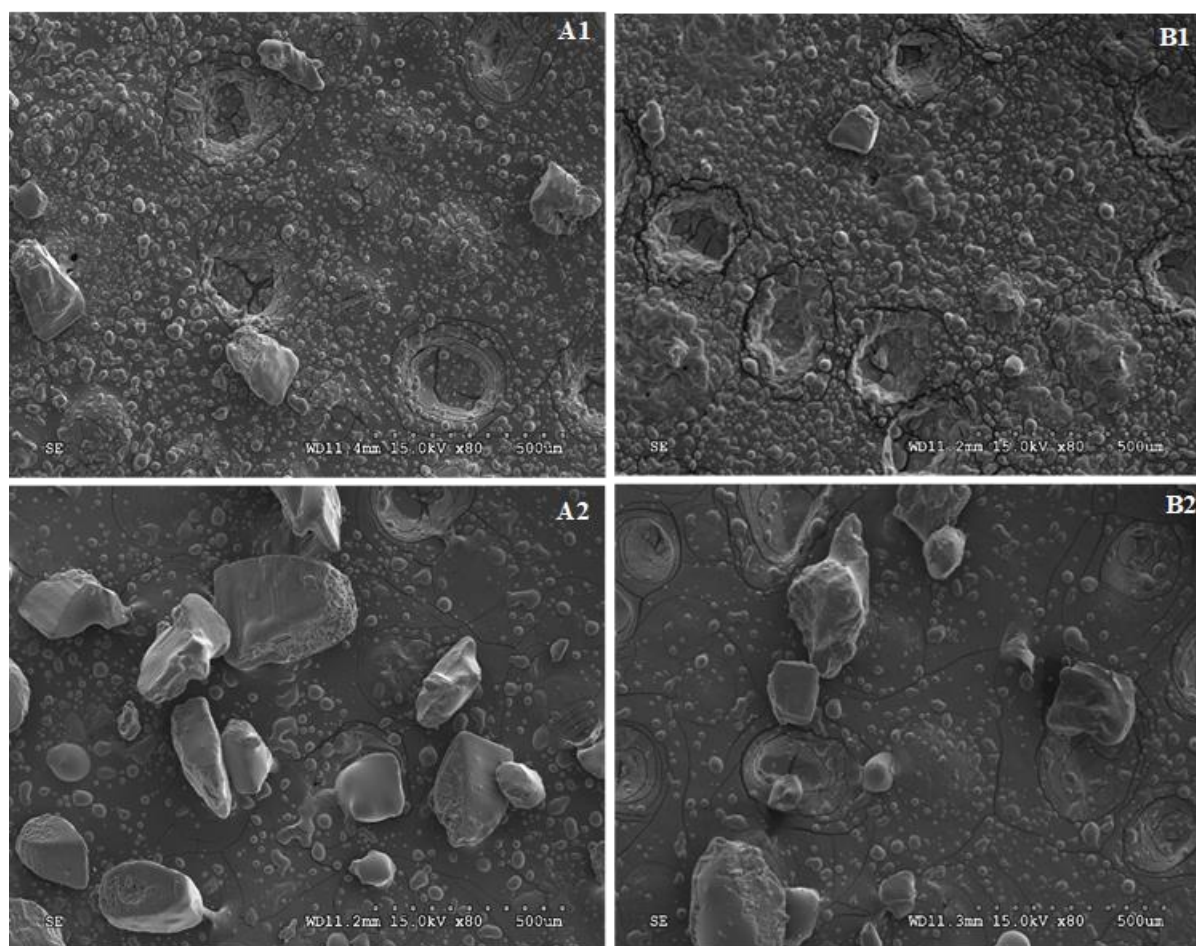
Scanning electron micrographs ( $\times 500$ ) of spray dried DPW samples containing low lactic acid (pH 6.1) high galactose (0.3%) and medium lactic acid (pH 5.55) high galactose (0.3%) before storage at 25°C are shown in Figure 4.10.



**Figure 4.10** Scanning electron micrographs ( $\times 500$ ) of spray dried deproteinized whey powder (A) High galactose (0.3%) low acid and (B) High galactose (0.3%) medium acid before storage at 25°C.

A variety of factors affect the microstructure of powders for instance composition of the powder and drying conditions such as temperature (Caric and Kalab, 1987). The same drying conditions with an inlet temperature of 180°C and outlet temperature of 83-87°C were used for spray drying of all powders. It was observed that the DPW powders right after production consisted of globular/spherical shaped particles and the powder particles were not attached to each other making it a non-hygroscopic free-flowing powder. It also had less round shaped particles with slight elongation and partly sharp-edged particles. Previously, Nishanthi et al. (2017) reported that sweet whey powder had large spherical particles when compared to native whey powder, acid whey powder, and salty whey powder. The powder particles of DPW powders were found to be pretty smooth on the outer surface. Similarly, smooth particles were observed by Mistry et al. (1992) in dried permeate powder containing 83% lactose and 4%

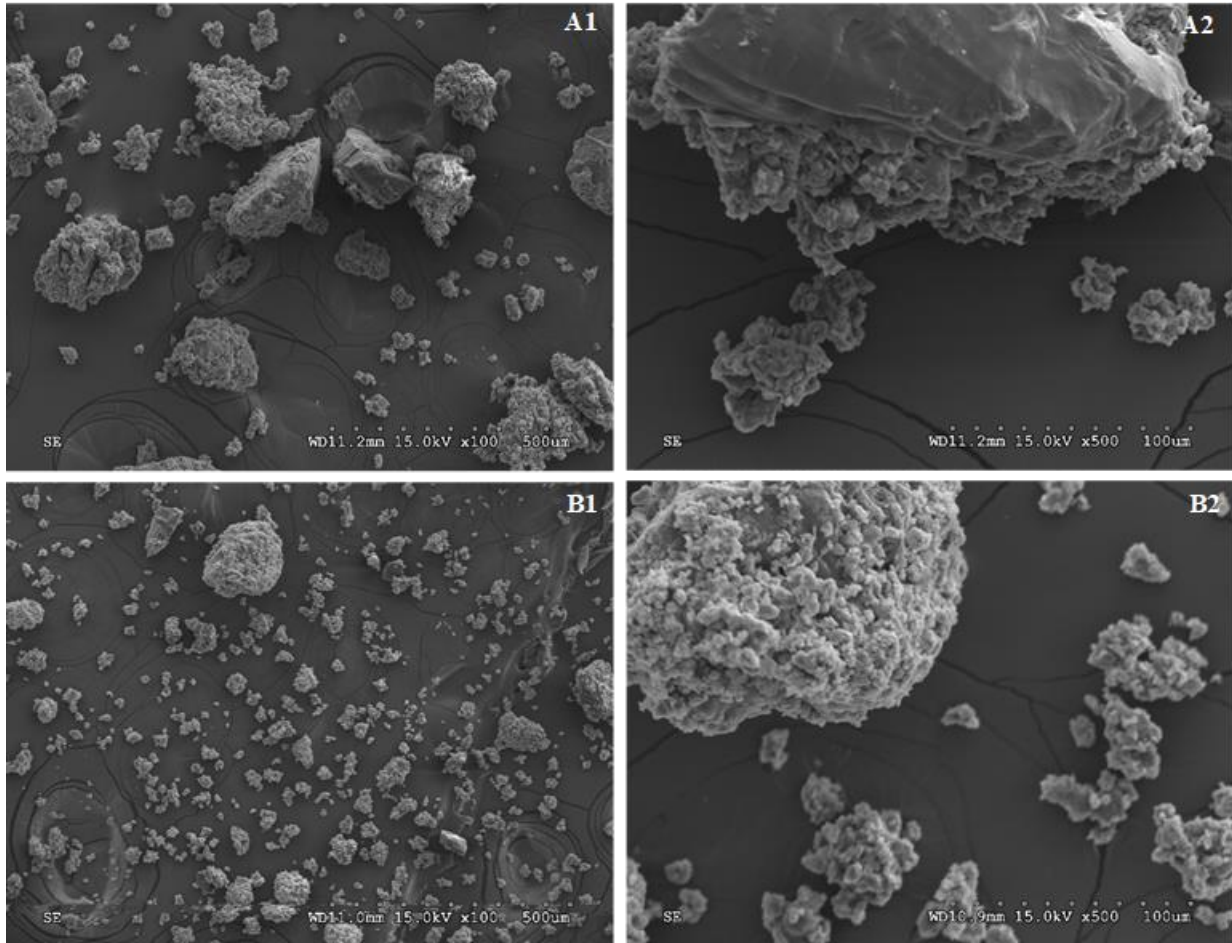
protein. They also suggested that lactose and protein influenced the powder particles. Kim et al. (2009) reported that the smooth surfaced particles are formed as a result of the high total solid content of the feed and high inlet temperature during spray drying. Micrographs of DPW powders before storage showed no evident wrinkles or dents on the DPW powder particles.



**Figure 4.11** Scanning electron micrographs ( $\times 80$ ) of spray dried deproteinized whey powder (A) low lactic acid (pH 6.1) high galactose (0.3%) and (B) medium lactic acid (pH 5.55) high galactose (0.3%) after storage at 25°C for (1) 90 and (2) 210 days.

Scanning electron micrographs ( $\times 80$ ) of spray dried DPW powders containing low lactic acid (pH 6.1) high galactose (0.3%) and medium lactic acid (pH 5.55) high galactose (0.3%) after storage at 25°C for 90 and 210 days are presented in Figure 4.11. The lactose in the powders started absorbing moisture which could be attributed to the increased water activity (Figure 4.1) during storage. Thus, the particles started coming together and can be seen as water

droplets that come together with some amount of caked particles as shown in Figure 4.10 on 90 days of storage. However, there was an increased number of caked particles by 210 days of storage.



**Figure 4.12** Scanning electron micrographs of spray dried deproteinized whey powder (A) low lactic acid (pH 6.1) high galactose (0.3%) and (B) medium lactic acid (pH 5.55) high galactose (0.3%) after storage at 25°C for 270 days with magnification (1)  $\times$  100 and (2)  $\times$  500.

The scanning electron micrographs of spray dried DPW powder samples containing low lactic acid (pH 6.1) high galactose (0.3%) and medium lactic acid (pH 5.55) high galactose (0.3%) after storage at 25°C for 270 days with magnification  $\times$  100 and  $\times$  500 are shown in Figure 4.12. The scanning electron microscopic (SEM) images on 270 days of storage had small particles stuck to the large caked particles. The rationale for this being that by 270 days of

storage the DPW powders were completely caked and for the sample used for SEM, it had to be broken with a mortar and pestle for analysis. Thus, the force applied to break the powder probably resulted in the distortion of the particles. The morphology of the particles became increasingly sticky, agglomerated, and caked as the storage days increased. Also, cluster-like particle agglomeration was observed as storage days increased attributing to the high moisture content.

## **Conclusions**

The findings of this study provide useful additional information on the effects of lactic acid and galactose on the physicochemical and thermal properties of DPW powders. Higher lactose contents, presence of impurities, Maillard reaction rate, storage time/temperature, and relative humidity can be considered as the predominant factors affecting the storage stability of DPW powders. Browning and formation of caked DPW powder has a negative impact on product quality. It is thus associated with additional costs as it can stop the production process or demands additional processing to regain a free-flowing powder.

## **References**

- Aguilera, J., J. del Valle, and M. Karel. 1995. Caking phenomena in amorphous food powders. *Trends Food Sci. Technol.* 6:149-155.
- AOAC International. 2002. Official methods of analysis. 17th ed. Association of Analytical Communities, Gaithersburg, MD.
- Banavara, D. S., D. Anupama, S. A. Rankin. 2003. Studies on physicochemical and functional properties of commercial sweet whey powders. *J. Dairy Sci.* 86:3866-3875.
- Bell, L. N. 1995. Kinetics of non-enzymatic browning in amorphous solid systems: Distinguishing the effects of water activity and the glass transition. *Food Res. Int.* 28:591-597.
- Birlouez-Aragon, I., J. Leclere, C. L. Quedraogo, E. Birlouez and J. Grongnet. 2001. The FAST method, a rapid approach of the nutritional quality of heat-treated foods. *Food/Nahrung.* 45:201-205.

- Buera, M. D. P., J. Chirife, S. L. Resnik. 1990. Nonenzymatic nonoxidative browning in hydrolyzed shelf-stable concentrated cheese whey. *J. Food Sci.* 55: 697-700.
- Burin, L., K. Jouppila, Y. Roos, J. Kansikas, and M. d. P. Buera. 2000. Color formation in dehydrated modified whey powder systems as affected by compression and Tg. *J. Agric. Food Chem.* 48:5263-5268.
- Caric, M. and M. Kalab. 1987. Effects of drying techniques on milk powders quality and microstructure: A review. *Food Structure.* 6:171-180.
- Carpin, M., H. Bertelsen, A. Dalberg, C. Roiland, J. Risbo, P. Schuck and R. Jeantet. 2017. Impurities enhance caking in lactose powder. *J. Food Eng.* 198:91-97.
- Carpin, M., H. Bertelsen, J. K. Bech, R. Jeantet, J. Risbo and P. Schuck. 2016. Caking of lactose: A critical review. *Trends Food Sci. Technol.* 53:1-12.
- Chandrapala, J., R. Wijayasinghe, and T. Vasiljevic. 2016. Lactose crystallization as affected by presence of lactic acid and calcium in model lactose systems. *J. Food Eng.* 178:181-189.
- Chandrapala, J. and T. Vasiljevic. 2017. Properties of spray dried lactose powders influenced by presence of lactic acid and calcium. *J. Food Eng.* 198:63-71.
- Chen, X. D. and N. Özkan. 2007. Stickiness, functionality, and microstructure of food powders. *Drying Technol.* 25:959-969.
- Dattatreya, A. and S. A. Rankin. 2006. Moderately acidic pH potentiates browning of sweet whey powder. *Int. Dairy J.* 16:822-828.
- Dattatreya, A., W. Lee and S. A. Rankin. 2010. Presence of galactose and glucose promotes browning of sweet whey powder. *J. Dairy Sci.* 93:2354-2357.
- Desic, S.D. and Birlouez-Aragon, I. 2011. The FAST index—A highly sensitive indicator of the heat impact on infant formula model. *Food chem.* 124:1043-1049.
- Eichner, K. and M. Karel. 1972. Influence of water content and water activity on the sugar-amino browning reaction in model systems under various conditions. *J. Agric. Food Chem.* 20:218-223.
- Ferrer, E., A. Alegría, R. Farré, G. Clemente and C. Calvo. 2005. Fluorescence, browning index, and color in infant formulas during storage. *J. Agric. Food Chem.* 53:4911-4917.
- Gombas, A., P. Szabó-Révész, M. Kata, G. Regdon and I. Erős. 2002. Quantitative determination of crystallinity of  $\alpha$ -lactose monohydrate by DSC. *J Therm. Anal. Calorim.* 68:503-510.
- Hargrove, R. E., F. E. McDonough, D. E. Lacroix and J. A. Alford. 1976. Production and properties of deproteinized whey powders. *J. Dairy Sci.* 59:25-33.
- Hartmann, M. and S. Palzer. 2011. Caking of amorphous powders—Material aspects, modeling and applications. *Powder Technol.* 206:112-121.
- Hooi, R., D. M. Barbano, R.L. Bradley, D. Budde, M. Bulthaus, M. Chettiar, J. Lynch, R. Reddy, E. A. Arnold. 2004. Chemical and physical methods. In: Wehr, M.H., Frank, J.F. editors.

- Standard methods for the examination of dairy products. 17<sup>th</sup> edition. American Public Health Association. Washington, D.C. 363-532.
- Huppertz, T. and I. Gazi. 2016. Lactose in dairy ingredients: Effect on processing and storage stability. *J. Dairy Sci.* 99:6842-6851.
- Jaya, S. and H. Das. 2004. Effect of maltodextrin, glycerol monostearate and tricalcium phosphate on vacuum dried mango powder properties. *J. Food Eng.* 63:125-134.
- Karmas, R., M. Pilar Buera and M. Karel. 1992. Effect of glass transition on rates of nonenzymic browning in food systems. *J. Agric. Food Chem.* 40:873-879.
- Kelly, P. M. 2009. Significance of Lactose in Milk Powders. Pages 80-97 in *Advanced Dairy Chemistry*. Vol. 3. P. McSweeney and P. F. Fox, ed. Springer, New York, NY.
- Kim, E. H., X. D. Chen and D. Pearce. 2009. Surface composition of industrial spray-dried milk powders. 2. effects of spray drying conditions on the surface composition. *J. Food Eng.* 94:169-181.
- Labuza, T. P. 1970. Water content and stability of low moisture and intermediate moisture foods. *Food Technol.* 24:543-550.
- Labuza, T. P. and M. Saltmarch. 1981. Kinetics of browning and protein quality loss in whey powders during steady state and nonsteady state storage conditions. *J. Food Sci.* 47:92-96.
- Le, T. T., B. Bhandari, H. C. Deeth. 2011. Chemical and physical changes of milk protein concentrate (MPC80) powder during storage. *J. Agric. Food Chem.* 59:5465-5473.
- Liogier de Sereys, A., Muller, S., Desic, S.D., Troise, A.D., Fogliano, V., Acharid, A., Lacotte, P. and Birlouez-Aragon, I. 2014. Potential of the FAST index to characterize infant formula quality. In *Handbook of dietary and nutritional aspects of bottle feeding*. Wageningen Academic Publishers. 653-656.
- Listiohadi, Y., J. Hourigan, R. Sleight, and R. Steele. 2005. An exploration of the caking of lactose in whey and skim milk powders. *Aust. J. Dairy Technol.* 60:207-213.
- Maltini, E., M. Anese and I. Shtylla. 1997. State diagrams of some organic acid-water systems of interest in food. *Cryo-Letters*.
- Maskan, M. 2001. Kinetics of colour change of kiwifruits during hot air and microwave drying. *J. Food Eng.* 48:169-175.
- Matiacevich, S. B. and M. P. Buera. 2006. A critical evaluation of fluorescence as a potential marker for the maillard reaction. *Food Chem.* 95:423-430.
- Mistry, V. V., H. N. Hassan, D. J. Robison. 1992. Effect of lactose and protein on the microstructure of dried milk. *Fd. Struct.*, 11:8.
- Naranjo, G. B., A. S. P. Gonzales, G. E. Leiva, L. S. Malec. 2013. The kinetics of Maillard reaction in lactose-hydrolysed milk powder and related systems containing carbohydrate mixtures. *Food Chem.* 141:3790-3795.

- Nishanthi, M., J. Chandrapala and T. Vasiljevic. 2017. Compositional and structural properties of whey proteins of sweet, acid and salty whey concentrates and their respective spray dried powders. *Int. Dairy J.* 74:49-56.
- Norwood, E., M. Chevallier, C. Le Floch-Fouéré, P. Schuck, R. Jeantet and T. Croguennec. 2016. Heat-induced aggregation properties of whey proteins as affected by storage conditions of whey protein isolate powders. *Food Bioproc. Technol.* 9:993-1001.
- Overend, W. G., A. R. Peacocke, and J. B. Smith. 1961. Reactions at position 1 of carbohydrates. part I. the polarographic reduction of carbohydrates. *J. Chem. Soc.* 3487-3497.
- Rao, R.D., Wendorff, W.L. and Smith, K., 2004. Changes in galactose and lactic acid content of sweet whey during storage. *J. Food Prot.* 67: 403-406.
- Roos, Y. H. 1993. Melting and glass transitions weight carbohydrates of low molecular. *Carbohydr. Res.* 238:39-48.
- Roos, Y. H. 2002. Importance of glass transition and water activity to spray drying and stability of dairy powders. *Le Lait.* 82: 475-484.
- Roos, Y. H., K. Jouppila and B. Zielasko. 1996. Non-enzymatic browning-induced water plasticization: Glass transition temperature depression and reaction kinetics determination using DSC. *J Therm. Anal. Calorim.* 47:1437-1450.
- Schebor, C., M. del Pilar Buera, M. Karel and J. Chirife. 1999. Color formation due to non-enzymatic browning in amorphous, glassy, anhydrous, model systems. *Food Chem.* 65:427-432.
- Schuck, P., A. Dolivet and R. Jeantet. 2012. *Analytical Methods for Food and Dairy Powders.* Wiley Online Library.
- Schuck, P., Dolivet, A. and Mejean, S. 2004. Lactose crystallization and hygroscopicity. *Advances in fractionation and separation: processes for novel dairy applications. Bulletin of the IDF,* 389: 10-15.
- Sithole, R., M. R. McDaniel and L. M. Goddik. 2005. Rate of Maillard browning in sweet whey powder. *J. Dairy Sci.* 88:1636-1645.
- Sithole, R., M. R. McDaniel and L. M. Goddik. 2006. Physicochemical, microbiological, aroma, and flavor profile of selected commercial sweet whey powders. *J. Food Sci.* 71:C157-C163.
- Teunou, E. and J. J. Fitzpatrick. 1999. Effect of relative humidity and temperature on food powder flowability. *J. Food Eng.* 42:109-116.
- Torres, J. K. F., R. Stephani, G. M. Tavares, A. F. De Carvalho, R. G. B. Costa, de Almeida, Carlos Eduardo Rocha, M. R. Almeida, de Oliveira, Luiz Fernando Cappa, P. Schuck and Í T. Perrone. 2017. Technological aspects of lactose-hydrolyzed milk powder. *Food Res. Int.* 101:45-53.

Wijayasinghe, R., T. Vasiljevic, J. Chandrapala. 2015. Water-lactose behavior as a function of concentration and presence of lactic acid in lactose model systems. *J Dairy Sci.* 98:8505-8514.



# **Chapter 5 - Effect of varying levels of lactic acid, galactose, and storage temperature on the physiochemical and thermal properties of milk permeate powders**

## **Abstract**

Milk permeate (MP) is the product obtained by removing milk proteins and milk fat from milk, partly skimmed milk, skimmed milk by ultrafiltration. It is then concentrated, crystallized, and dried using spray drying techniques to get MP powder which has a slightly salty taste and uniform particle size. MP powders typically contain 78-88% lactose, 3-5% protein, 3-4.5% moisture, and 8-11% ash. MP powder is widely used to standardize skim milk powder and is suitable for a wide range of applications including beverages, seasonings, confectionery, soups, sauces, desserts, and baked goods. However, the increased amounts of lactose can cause Maillard browning, stickiness, and caking which can be detrimental for storage stability. This study aimed at investigating the effect of different levels of lactic acid and galactose on the physiochemical and thermal properties of MP powders. For this study, two lots of MP powders were procured from a commercial manufacturer and were reconstituted to 45% total solids with water. The samples were divided into 4 subsamples with treatment combinations of high and low levels of lactic acid by adjusting the pH to 6.0 by adding lactic acid and pH 6.26 with no addition and galactose (0 and 0.30%) as per the experimental design. The samples were dried in a lab scale spray dryer with inlet and outlet temperatures of 180 and 85°C, respectively. The spray dried MP powders were sealed and stored at 25°C (for 0 (control), 60, and 120 days) and 40°C (for 0 (control), 30, 60, 90, 120, and 150 days). At each data point, the powders were analyzed for water activity ( $a_w$ ), color, browning index (BI), FAST index (FI), hygroscopicity, caking, differential

scanning calorimetry (DSC), and microstructure. In MP powders, the main effect lactic acid was significant for  $a_w$ ,  $a^*$ , FI and hygroscopicity at 25°C whereas at 40°C the main effect lactic acid was significant for  $a_w$ ,  $a^*$ ,  $b^*$ , BI and FI. It was found that the  $a_w$ , BI, and hygroscopicity increased significantly during storage. Overall, all powders had a tendency to absorb moisture during storage, however, the powders with higher amounts of lactic acid and galactose were more sensitive to absorbing moisture, giving rise to lumping and caking problems. These findings emphasize the importance of controlling the presence of lactic acid and galactose and the storage conditions to limit caking and to enhance storage stability.

Keywords: Milk permeate powder, lactic acid, galactose, storage temperature, storage time

## **Introduction**

Lactose is the major carbohydrate in milk and one of the main components of permeate powders. Milk permeates are coproducts obtained during the ultrafiltration of milk. Subsequently, the milk permeates are subjected to unit operations like evaporation, crystallization, and spray drying resulting in a non-hygroscopic, free flowing powder. Milk permeate (MP) powder is a coproduct obtained from the production of milk protein concentrate, milk protein isolate, and ultrafiltered milk manufacturing. MP powders generally consist of 78-88% lactose, 3-5% protein, 3-4.5% moisture, 0-1.0% fat, and 8-11% ash. MP has a comparable composition to whey permeate and since it is derived straight from milk, it has fewer processing steps. MP powders are known for its clean, slightly salty taste, uniform particle size and has applications in baked goods, soups, sauces, confectioneries, dry mixes, meats, dairy foods, and beverages. MP powders can also be used to standardize skim milk powder and are considered as a natural food ingredient with excellent functional and nutritional characteristics. Permeate also

has an umami effect that escalates flavors and allows reduced usage of other specific flavor ingredients.

Stickiness and caking are common undesirable problems in high lactose dairy ingredients due to the presence of lactose. It is, therefore, crucial to understand the mechanisms that cause sticking between particles and how processing/storage conditions affect the rate of stickiness development in these powders. Furthermore, the moisture content and water activity determine mobility and reactivity which induces Maillard reactions, color development, and caking phenomena. Bronlund and Paterson (2004) noted that the water sorption of crystalline lactose shows no noticeable temperature dependence between 12 to 37°C. Strong correlations between glass transition temperature ( $T_g$ ) and stickiness have been widely studied (Schuck et al., 2005; Fitzpatrick et al., 2007; Hogan and O'Callaghan, 2013; Silalai and Roos, 2010). Preventing the caking of permeate powders during storage is essential to retain desired handling properties. Sufficiently low moisture contents combined with controlled storage conditions have been suggested to prevent or minimize caking during storage (Aguilera et al., 1995; Listiohadi et al., 2005; Kelly, 2009). The caking in crystalline lactose powder has been reviewed by Carpin et al. (2016). Caking is known reduce flowability (Juliano and Barbosa-Canovas, 2010).

The presence of the crystalline state and/or a thermodynamically unstable amorphous state influences the physical properties (Kedward et al., 2000). Dec and Chojnowski (2006) reported that the presence of lactic acid can cause caking of whey powder particles during spray drying of acid whey. Lactic acid is highly hygroscopic, thus may impact sorption behavior, which in turn, influence the processability of acid whey (Chandrapala et al., 2017). The quality of dairy ingredients deteriorates by physical processes like crystallization or recrystallization of lactose as

functionality is reduced (Stapelfeldt et al., 1997). The rate and extent of these processes and reactions is critical to maintain the overall product quality (Thomsen et al., 2005).

Changes in the storage stability of MP powder due to varying levels of lactic acid and galactose have not been studied previously. Also, the time/temperature dependence on the storage stability of MP powders were also not been previously examined. The objective of this study is to investigate the effect of the presence of impurities (lactic acid and galactose) and temperature on the storage stability of MP powders.

## **Materials and methods**

### **Experimental design**

The MP powder (2 lots) were collected from a commercial manufacturer within the United States. The MP powders (2 lots) were reconstituted to 45% total solids with cold water (~4°C) so that the crystallized lactose does not dissolve, and this was divided into 4 subsamples with varying levels of lactic acid and galactose. The treatment combinations were such that the high lactic acid (by adjusting pH to 6 with lactic acid) and low lactic acid (no addition) and galactose (0 and 0.3%) as per the experimental design. The treatment with low lactic acid (pH 6.26) low galactose (0%) was considered as control. Each of the samples were spray dried and sealed in poly lined kraft bags (Proliant Dairy, USA). The samples were stored at 25°C for 0 (control), 60, and 120 days and 40°C for 0 (control), 30, 60, 90, 120, and 150 days in an incubator (Hettcube 200R, Beverly, MA, USA). On the designated day, the powders were analyzed for water activity ( $a_w$ ), color, browning index (BI), FAST index (FI), hygroscopicity, caking, differential scanning calorimetry (DSC), and microstructure. All the measurements were carried out in duplicates.

## **Laboratory-scale production of powders and packaging**

The MP (2 lots) were spray dried using a pilot scale spray dryer (Y-105, Shanghai, China) at an inlet air temperature of 180°C and the outlet air temperature was maintained between 85-88°C, feed temperature less than 20°C, and a pump rate of 7. The RH of the room was periodically recorded using a digital humidity meter (Traceable Humidity Meter, Fisher Scientific, Hampton, NH, USA).

## **Composition**

The moisture content of DPW powders was determined in duplicate using the oven method (IDF, 1993). The drying temperature was  $103 \pm 1^\circ\text{C}$ , and the drying time was 4 h. Total nitrogen of MP powders were analyzed using AOAC (2016) standard methods (990.20). Ash content was determined after the ignition of the sample at 550°C (AOAC International, 2002; method 954.46). A 5% (w/w) solution of MP powder was prepared for measuring pH. The pH measurements were done with an Accumet® portable AP63 pH/mV meter (Fisher Scientific, Pittsburgh, PA, USA) calibrated with standardized buffer solutions (pH 4, 7, and 10) before the measurement. Titratable acidity (% lactic acid) was done using the method described by Hooi et al. (2004).

## **Water activity**

The water activity of the MP powders was measured using the Aqua Lab CX-2 (Decagon Devices Inc., Pullman, WA, USA) water activity meter.

## **Color**

The  $L^*$ ,  $a^*$ , and  $b^*$  values of the MP powders were measured using HunterLab, Miniscan XE (Reston, VA).  $L^*$  corresponds to whiteness (0 - 100),  $a^*$  corresponds to green to red on the positive-to-negative scale, respectively, and  $b^*$  corresponds to blue to yellow on the positive-to-

negative scale, respectively. The instrument was standardized using black and white tiles. A plastic petri dish containing the sample (4 mm thickness) was placed above the light source and the  $L^*$ ,  $a^*$ , and  $b^*$  values were recorded.

### **Browning Index**

The browning index (BI) of the MP powders were determined according to Maskan, (2001) using  $L^*$ ,  $a^*$ ,  $b^*$  values from the colorimeter (HunterLab, Miniscan XE, Reston, VA. The BI was determined by the following equation:

$$BI = \frac{100 (x - 0.31)}{0.17}$$

where  $x = \frac{a^* + 1.75 * L^*}{5.645 * L^* + a^* - 3.012 * b^*}$

### **FAST Index**

The Perkin Elmer LS-55 (PerkinElmer Life and Analytical Sciences, Waltham, MA, USA) fluorimeter equipped with a xenon lamp and excitation and emission monochromators were used to obtain the fluorescence emission spectra of tryptophan (Trp) and Maillard reaction products spectra. Trp fluorescence was measured at 290/340 nm. Fluorescence of advanced Maillard products (AMP) was measured at 330/420 nm. Each MP powder sample was analyzed 3 times. The data was averaged and the peak intensities were used to calculate the FAST Index (FI) values. The FI was calculated by the following equation:

$$FI = \frac{F_{AMP}}{F_{TRP}} \times 100$$

### **Hygroscopicity**

The hygroscopicity of the MP powders were measured by the method described by Jaya and Das (2004) with slight modifications. The MP powder samples (1 g) was placed in aluminum pans (Fischer Scientific, Pittsburgh, PA, USA), which were then equilibrated in

desiccators containing saturated solution with a known relative humidity of sodium chloride (RH=75% at 25 °C). Samples were kept at controlled temperature of 25°C and after 24h weighed each sample up to the weight difference of no more than 0.5%. Hygroscopicity was measured through the equation:

$$Hy = \frac{((w2 - w1 - w0) \times 1000) + (w1 \times M)}{(w2 - w0) \times 10}$$

where w0 is the weight (in grams) of the dish, w1 is the weight (in grams) of the sample, w2 is the weight (in grams) of the dish after equilibrium has been reached and M is the moisture content of the sample, in grams per kilogram (Hargrove et al., 1976; Schuck et al., 2012).

### **Caking**

The determination of the caking of MP powders was done by visual examination and the images were captured with camera (12-megapixel sensor with an f/1.7 aperture).

### **Differential scanning calorimetry (DSC)**

Thermal properties of MP powders were determined using a differential scanning calorimeter DSC Q2000 (TA Instruments, New Castle, DE, USA) equipped with thermal analysis software (TA Instrument Control). The instrument was calibrated using indium. MP powder (4.5 mg) was directly placed in the aluminum pan, hermetically sealed, and scanned for temperature from 25 to 220 °C at 5°C/min. An empty pan was used as a reference. Peak temperature and enthalpy were computed from the thermograms using the data processing software.

### **Microstructure**

The microstructure of MP powders were examined using a scanning electron microscope. The imaging was conducted using a S-3500N (Hitachi Science Systems Ltd., Tokyo, Japan) and examined by a secondary electron detector operating at 10 kV. The MP powders were directly

mounted onto a carbon double-sided adhesive tape on microscopy stubs and sputter coated with palladium using a Denton Vacuum Desk II sputter coater (Denton Vacuum, Moorestown, NJ) for 15 min to avoid the charge buildup under the electron beam.

### **Statistical Analysis**

Statistical analyses were performed using the procedure PROC GLM of SAS software (SAS version 9.2, SAS Institute Inc., Cary, NC), which involved 2 factors (lactic acid and galactose) each with 2 levels (high and low). A repeated measures experimental design, with a two-way whole-plot treatment structure, in a completely randomized whole-plot design structure was used. Repeated measurements were taken over time. Lactic acid and galactose, the whole-plot factors, were applied in a  $2 \times 2$  factorial treatment structure. A probability of  $p < 0.05$  was used to establish statistical significance for fixed effects and interactions. All the experimental parameters were measured in duplicates. The data points are reported as mean  $\pm$  standard deviation. All statistical analyses were performed with SAS software. Significantly different groups were compared using PROC GLM in SAS (version 9.4, SAS Institute Inc., Cary, NC) by Tukey's test at a significance when  $p < 0.05$ .

## **Results and discussion**

### **Composition**

MP powder composition is summarized in Table 5.1. All samples were within specification for protein and moisture (American Dairy Products Institute). The probability values from ANOVA of the effects of different lactic acid concentrations, galactose concentrations, and storage days on  $a_w$ , color ( $L^*$ ,  $a^*$ ,  $b^*$ ), BI, FAST index, and hygroscopicity of milk permeate powders during storage at 25°C and 40°C are shown in Table 5.2 and Table 5.3, respectively.



**Table 5.1** Composition of milk permeate powders

Samples	Total Protein (%)	Moisture (%)	Ash (%)	pH	Titrateable acidity (%)
L1G1	2.71±0.05	1.33±0.03	7.55±0.68	6.26	0.04
L1G2	2.32±0.23	1.59±0.04	6.63±0.42	6.23	0.04
L2G1	2.36±0.09	1.49±0.14	6.90±0.39	6.19	0.05
L2G2	2.30±0.18	1.50±0.07	7.06±0.24	6.17	0.06

Results are the means ± standard deviations of data from two independent trials (two lots)  
 \*Treatments: L1G1 = low lactic acid low galactose; L1G2 = low lactic acid high galactose;  
 L2G1 = high lactic acid low galactose; L2G2 = high lactic acid high galactose.

**Table 5.2** Probability values from ANOVA of the effects of different lactic acid concentrations, galactose concentrations, and storage days on  $a_w$ , color ( $L^*$ ,  $a^*$ ,  $b^*$ ), browning index, FAST index, and hygroscopicity of milk permeate powders during storage at 25°C

Source**	NDF	$a_w$	$L^*$	$a^*$	$b^*$	BI	FI	Hy
LA	1	0.0005*	0.2233	0.0414*	0.8871	0.5298	0.0001*	0.0004*
GA	1	0.0601*	0.5945	0.3607	0.9055	0.7503	0.0005*	0.2700
LA x GA	1	0.7707	0.8227	0.8093	0.8976	0.8963	0.7453	0.0185*
Time	5	0.0001*	0.0001*	0.0001*	0.0002*	0.0001*	0.0001*	0.0001*
Time x LA	5	0.0001*	0.5597	0.8832	0.9389	0.8711	0.0001*	0.9266
Time x GA	5	0.1053	0.7125	0.9877	0.9566	0.9598	0.6062	0.5588
Time x LA x GA	5	0.0365*	0.7328	0.9800	0.9015	0.8782	0.0040*	0.7990

NDF is the numerator degrees of freedom of the approximate tests, the F tests.

\*The F test was significant at the 0.05 level.

$a_w$  = water activity, BI = browning index, FI = FAST index, Hy = hygroscopicity

\*\*LA – lactic acid, GA – galactose

**Table 5.3** Probability values from ANOVA of the effects of different lactic acid concentrations, galactose concentrations, and storage days on  $a_w$ , color ( $L^*$ ,  $a^*$ ,  $b^*$ ), browning index, FAST index, and hygroscopicity of milk permeate powders during storage at 40°C

Source**	NDF	$a_w$	$L^*$	$a^*$	$b^*$	BI	FI	Hy
LA	1	0.0049*	0.1179	0.0019*	0.0281*	0.0048*	0.0001*	0.7823
GA	1	0.1691	0.6956	0.1122	0.3043	0.1709	0.0002*	0.9726
LA x GA	1	0.9738	0.9987	0.5683	0.8753	0.9439	0.3482	0.9473
Time	5	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0052*
Time x LA	5	0.0127*	0.3419	0.0006*	0.0006*	0.0001*	0.0001*	0.9992
Time x GA	5	0.7468	0.9516	0.8783	0.7803	0.6572	0.0244*	0.9608
Time x LA x GA	5	0.1094	0.9637	0.9505	0.7552	0.8769	0.0084*	0.9670

NDF is the numerator degrees of freedom of the approximate tests, the F tests.

\*The F test was significant at the 0.05 level.

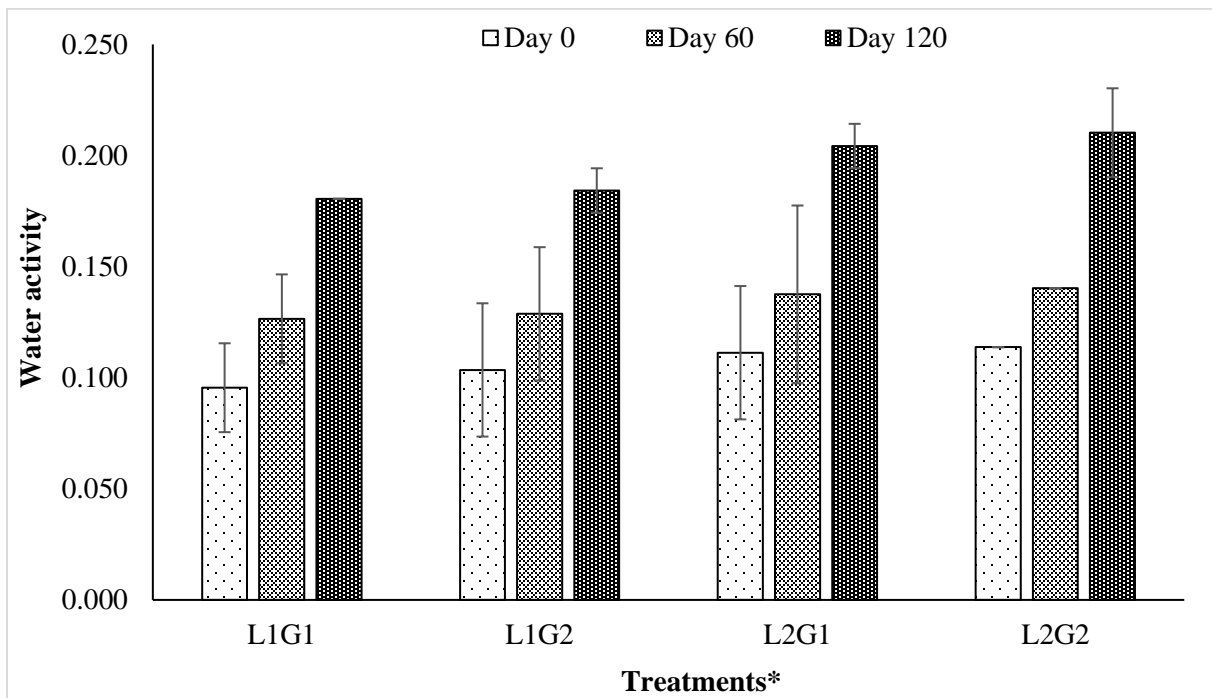
$a_w$  = water activity, BI = browning index, FI = FAST index, Hy = hygroscopicity

\*\*LA – lactic acid, GA – galactose

### Water activity

The  $a_w$  of MP powders right after production (fresh powders) were in the range of 0.096 to 0.114 which were found to be significantly ( $p < 0.05$ ) different. A previous study on spray dried MP powders reported  $a_w$  value of 0.11 which were close to the results obtained for fresh MP powders in our study (Shrestha et al., 2008). In fresh powders, the lowest  $a_w$  of 0.096 was for the sample containing low lactic acid (pH 6.26) low galactose (0%) whereas the highest  $a_w$  of 0.114 was for the sample containing high lactic (pH 6) acid high galactose (0.3%). Chandrapala and Vasiljevic (2017) reported an increase in  $a_w$  with increasing lactic acid concentrations in spray dried lactose powders. The  $a_w$  increased from 0.328 (lactose powder) to 0.392 (lactose + 0.2% lactic acid powder). The  $a_w$  was observed as 0.096 for control (low lactic acid (pH 6.26) low galactose (0%)) and 0.111 for high lactic acid (pH 6) low galactose (0%). The high lactic acid in our study meant 0.2% lactic which was the same as their study. Figure 5.1 illustrates the  $a_w$  values of MP powders during storage at 25°C for 0, 60, and 120 days. The  $a_w$  increased with

increasing storage time at 25°C for each treatment and was found to be significantly ( $p < 0.05$ ) different for all storage days. It was observed that the main effects lactic acid and galactose were found to have a significant ( $p < 0.05$ ) effect on  $a_w$  values. Also, time and the interaction effects time x lactic acid, and time x lactic acid x galactose each were also found to have a significant ( $p < 0.05$ ) effect on  $a_w$  values on the storage of MP powders at 25°C (Table 5.2). The  $a_w$  values of MP powders on 120 days of storage were in the range 0.179 to 0.210 with the lowest being for the sample containing low lactic acid (pH 6.26) low galactose (0%) and the highest for the sample containing high lactic acid (pH 6) high galactose (0.3%).



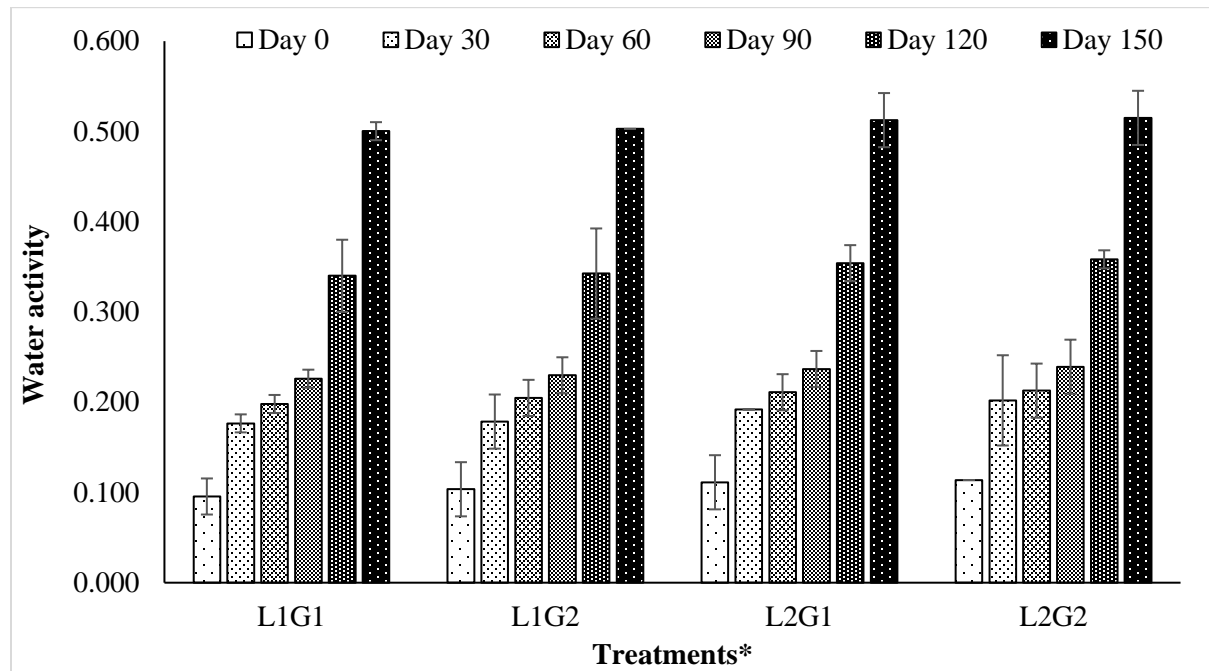
**Figure 5.1** Water activity values of milk permeate powders during storage at 25°C for 0, 60, and 120 days.

Error bars are standard deviations from two independent trials (two lots).

\*Treatments: L1G1 = low lactic acid low galactose; L1G2 = low lactic acid high galactose; L2G1 = high lactic acid low galactose; L2G2 = high lactic acid high galactose.

The  $a_w$  increased with increasing storage time for each treatment which were significantly ( $p < 0.05$ ) different for all the days of storage. Results showed that the  $a_w$  values were significantly ( $p < 0.05$ ) affected by the main effect lactic acid. It was observed that during

storage of MP powders at 40°C the  $a_w$  values were affected by time and the interaction effect time x lactic acid. The  $a_w$  values on 150 days of storage were in the range of 0.500 to 0.515.



**Figure 5.2** Water activity values of milk permeate powders during storage at 40°C for 0, 30, 60, 90, 120, and 150 days.

Error bars are standard deviations from two independent trials (two lots).

\*Treatments: L1G1 = low lactic acid low galactose; L1G2 = low lactic acid high galactose; L2G1 = high lactic acid low galactose; L2G2 = high lactic acid high galactose.

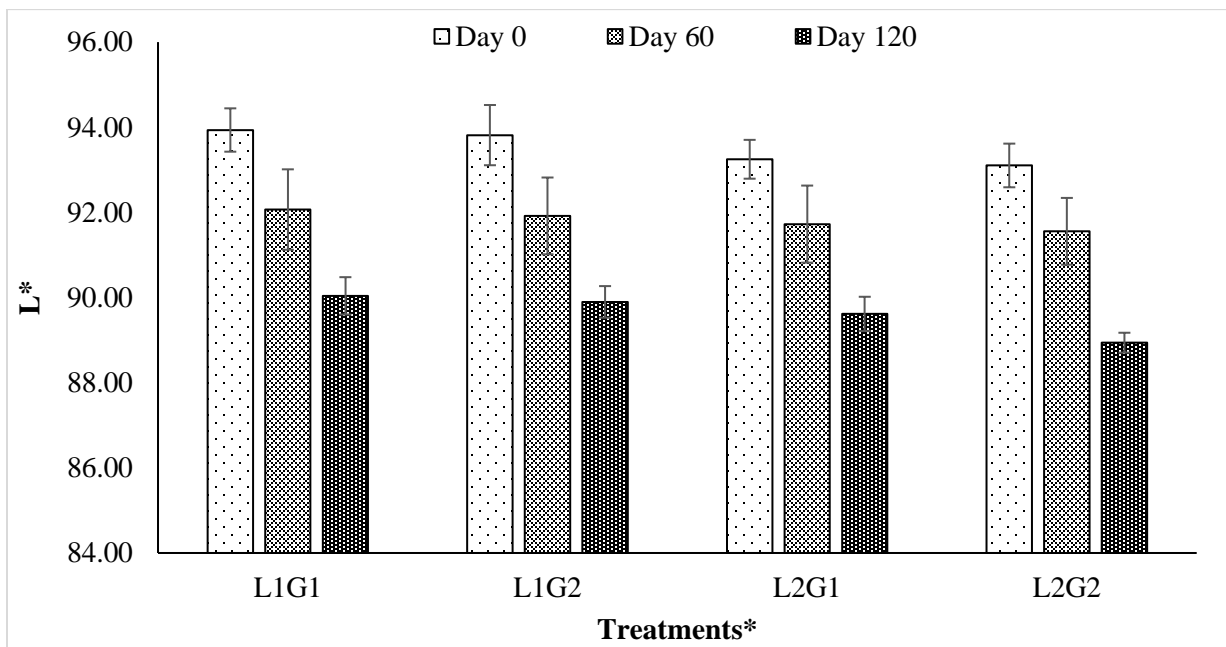
Jouppila and Roos (1994) reported that skim milk powder with hydrolyzed lactose had a lower  $T_g$  than regular due to the lower  $T_g$  of glucose and galactose of 31°C and 30°C, respectively. Lactic acid is a highly hygroscopic compound with a very low  $T_g$  value, approximately -60 °C (Maltini et al., 1997). Thus, an increase in  $a_w$  for the fresh powders with the presence of lactic acid and galactose could be attributed to the hygroscopic nature of lactic acid and low  $T_g$  of galactose, leading to absorbing more water molecules (Shrestha et al., 2007).

## Color

The  $L^*$ ,  $a^*$  (data not shown), and  $b^*$  values of MP powders right after production were in the range 93.94 to 93.10, -2.71 to -2.48, and 13.99 to 14.31, respectively. The  $L^*$ , and  $b^*$  values

were not significantly ( $p > 0.05$ ) different, however, the  $a^*$  values were found to be significantly ( $p < 0.05$ ) different for fresh powders. Shrestha et al. (2008) reported the  $L^*$ ,  $a^*$ , and  $b^*$  values of MP powders to be  $98.6 \pm 0.5$ ,  $-3.9 \pm 0.2$ , and  $10.0 \pm 0.2$ , respectively. The  $L^*$  values in this research were lower and  $b^*$  values were higher when compared to those reported by Shrestha et al. (2008).

$L^*$ . Figure 5.3 illustrates the  $L^*$  values of MP powders during storage at 25°C for 0, 60, and 120 days.

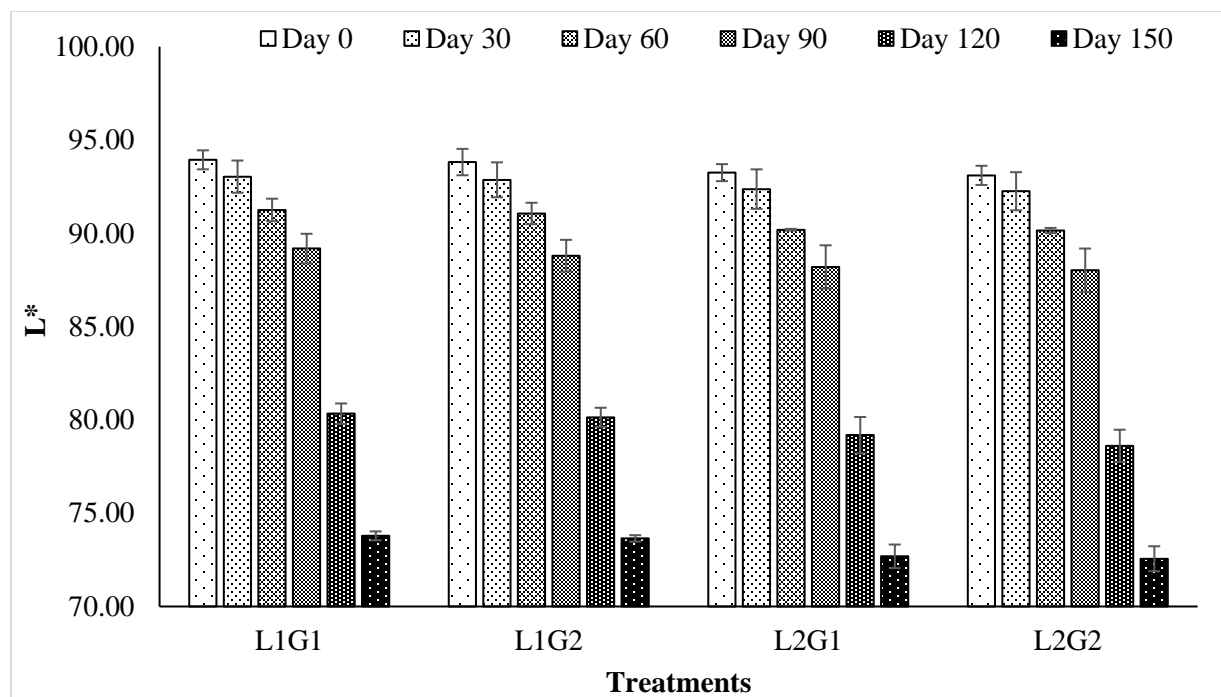


**Figure 5.3**  $L^*$  values of milk permeate powders during storage at 25°C for 0, 60, and 120 days

Error bars are standard deviations from two independent trials (two lots).

\*Treatments: L1G1 = low lactic acid low galactose; L1G2 = low lactic acid high galactose; L2G1 = high lactic acid low galactose; L2G2 = high lactic acid high galactose

In general, it was observed that the  $L^*$  values decreased gradually until 120 days of storage which was not significantly ( $p > 0.05$ ) different. The  $L^*$  values were only significantly ( $p < 0.05$ ) affected by time on the storage of MP powders at 25°C (Table 5.2). The  $L^*$  values of MP powders on 60 and 120 days of storage at 25°C were in the range 91.56 to 92.07 and 88.94 to 90.04, respectively.



**Figure 5.4** L\* values of milk permeate powders during storage at 40°C for 0, 30, 60, 90, 120, and 150 days.

Error bars are standard deviations from two independent trials (two lots).

\*Treatments: L1G1 = low lactic acid low galactose; L1G2 = low lactic acid high galactose; L2G1 = high lactic acid low galactose; L2G2 = high lactic acid high galactose.

Figure 5.4 illustrates the L\* values of MP powders during storage at 40°C for 0, 30, 60, 90, 120, and 150 days. In general, it was observed that the L\* values decreased gradually until 30 days of storage however it showed a substantial decrease during storage days 60, 90, 120 and 150 which were significantly ( $p < 0.05$ ) different. The L\* values were only significantly ( $p < 0.05$ ) affected by time on the storage of MP powders at 40°C (Table 5.3). The highest L\* value of 73.79 was found to be for the sample containing low lactic acid (pH 6.26) low galactose (0%) whereas the lowest L\* value of 72.56 was for the sample containing high lactic acid (pH 6) high galactose (0.3%) on 150 days of storage at 40°C.

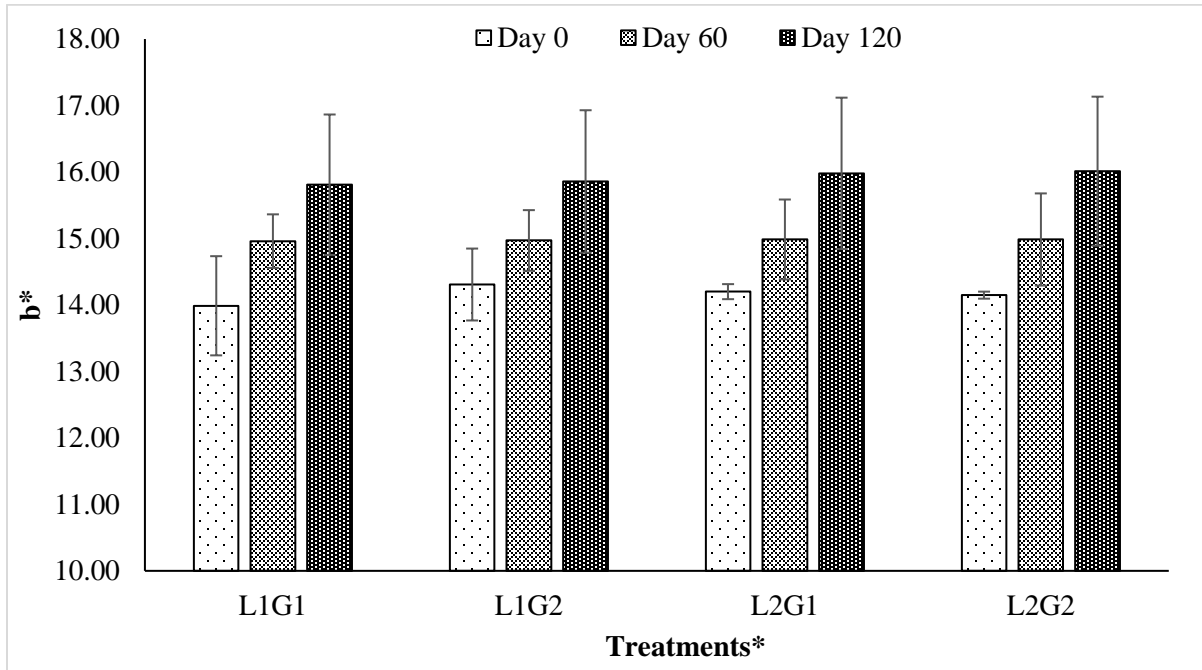
The L\* values were observed to be highest for the sample containing low lactic acid (pH 6.26) low galactose (0%) and the lowest for the sample containing high lactic acid (pH 6) high galactose (0.3%) for the MP powders stored at 25°C and 40°C. This could be attributed to the

presence of lactic acid and galactose present in these samples. Dattatreya et al. (2010) reported that the samples which contain galactose (1.5 g/L) has a greater degree of browning when compared to those containing just lactose (3.0 g/L) indicating that residual sugars have a significant effect on the browning in sweet whey powders. In our study, the galactose content used was 0.15% (0.675g/L) and 0.30% (1.35 g/L) compared to 1.5 g/L used in their study. The rationale for this being that the presence of sugars such as galactose could increase the initial rate of reducing sugar- dependent browning when it reacts with a given amino compound primarily by increasing the rate at which the sugar's ring opens to a reactive, reducible form (Overend et al., 1961; Dattatreya et al., 2010). In MP powders, it was observed that the L\* values decreased with decreasing pH. Similar results were obtained by Dattatreya and Rankin (2006) during the accelerated storage of sweet whey powder. They also observed that with the increase in temperature the L\* values were found to decrease and to reach an L\* value of 60, it took 6 months at 40°C, 8 days at 60°C whereas only 12 hours at 80°C.

**b\***. The b\* values of MP powders during storage at 25°C for 0, 60, and 120 days is shown in Figure 5.5. It was observed that the b\* values increased gradually with storage until 120 days of storage at 25°C which was not significantly ( $p > 0.05$ ) different. On storage at 25°C, the b\* values were only significantly ( $p < 0.05$ ) affected by the time (Table 5.2). The b\* values on 120 days of storage at 25°C were in the range 15.81 to 16.01.

The b\* values of MP powders during storage at 40°C for 0, 30, 60, 90, 120, and 150 days is shown in Figure 5.6. It was observed that the b\* values increased gradually with storage until 30 days of storage which was not significantly ( $p > 0.05$ ) different. The b\* values increased significantly ( $p < 0.05$ ) on 90, 120, and 150 days of storage at 40°C. The b\* values were significantly ( $p < 0.05$ ) affected by the main effect lactic acid. It was also significantly ( $p < 0.05$ )

affected by time and the interaction effect time x lactic acid on the storage of MP powders at 40°C (Table 5.3). The  $b^*$  values for MP powders on 150 days of storage at 40°C were in the range 19.55 to 20.91.



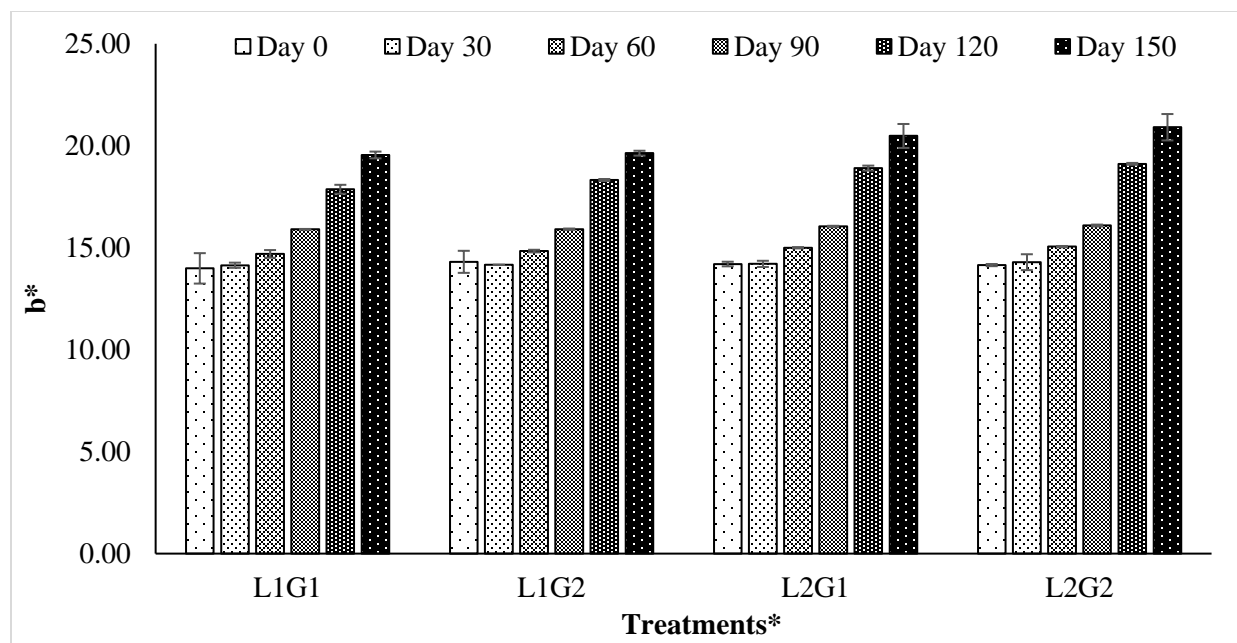
**Figure 5.5**  $b^*$  values of milk permeate powders during storage at 25°C for 0, 60, and 120 days.

Error bars are standard deviations from two independent trials (two lots).

\*Treatments: L1G1 = low lactic acid low galactose; L1G2 = low lactic acid high galactose; L2G1 = high lactic acid low galactose; L2G2 = high lactic acid high galactose.

It was observed that the  $b^*$  values increased with increasing storage time and temperature (25°C and 40°C) for the MP powders. The increase in  $b^*$  values could be also attributed to the presence of lactic acid and galactose however galactose did not have a significant effect at both storage temperatures. Previous literature suggests that the rate of deteriorative reactions and storage stability are related to the water activity of foods with the maximum rate of non-enzymatic browning reaction reported to take place in the  $a_w$  range of 0.5-0.75 (Gonzales et al., 2010; Kaanane and Labuza, 1989).





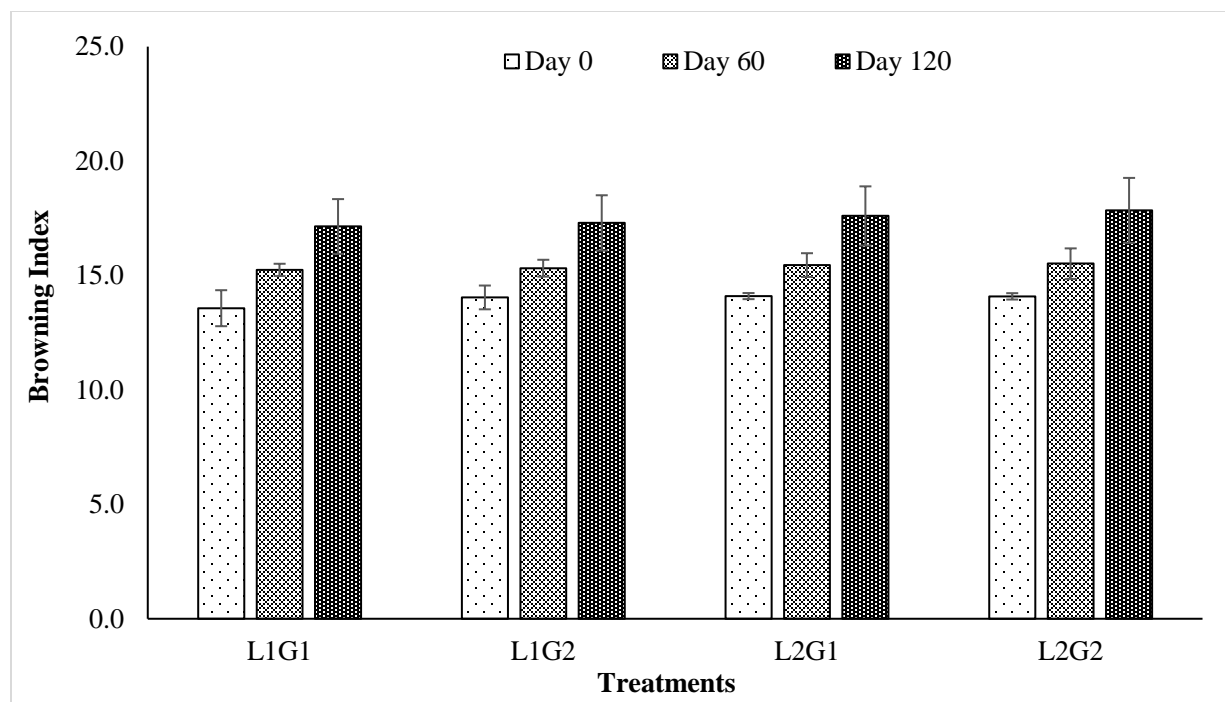
**Figure 5.6**  $b^*$  values of milk permeate powders during storage at 40°C for 0, 30, 60, 90, 120, and 150 days.

Error bars are standard deviations from two independent trials (two lots).

\*Treatments: L1G1 = low lactic acid low galactose; L1G2 = low lactic acid high galactose; L2G1 = high lactic acid low galactose; L2G2 = high lactic acid high galactose.

### **Browning Index**

Nonenzymatic browning of high lactose powders has been associated with reactions taking place between lactose and proteins called Maillard browning/ reaction on prolonged storage conditions. The BI values of spray dried MP powders right after production was found to be in the range 13.6 to 14.1 (AU) although it was not significantly ( $p > 0.05$ ) different. The BI values of MP powders during storage at 25°C for 0, 60, and 120 days is shown in Figure 5.7. The BI values gradually increased with increasing storage time; however, it was not significantly ( $p > 0.05$ ) different. The BI values were only significantly ( $p < 0.05$ ) affected by time for MP powders during storage at 25°C (Table 5.2). The BI values of MP powders during storage at 25°C for 120 days were observed to be in the range 17.1 to 17.8 (AU). The highest BI value of 17.8 on 120 days of storage was for the sample high lactic acid high (pH 6) galactose (0.3%).

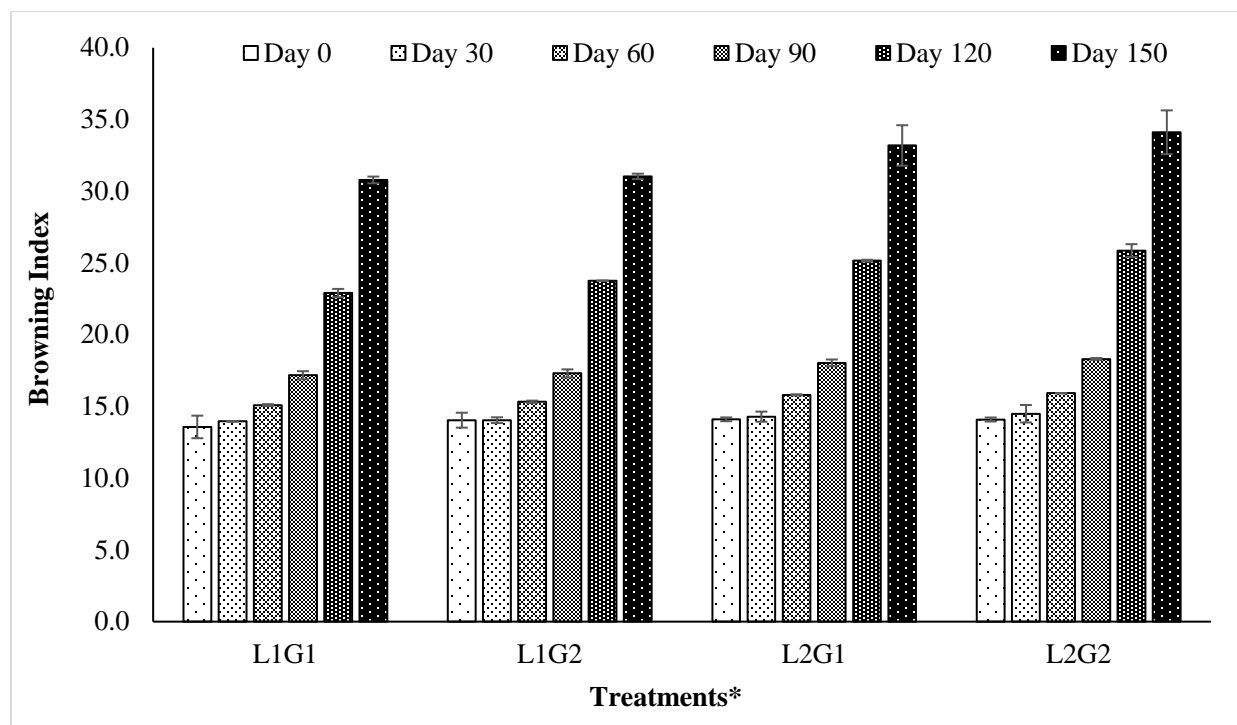


**Figure 5.7** Browning index values of milk permeate powders during storage at 25°C for 0, 60, and 120 days.

Error bars are standard deviations from two independent trials (two lots).

\*Treatments: L1G1 = low lactic acid low galactose; L1G2 = low lactic acid high galactose; L2G1 = high lactic acid low galactose; L2G2 = high lactic acid high galactose.

Figure 5.8 shows the BI values of MP powders during storage at 40°C for 0, 30, 60, 90, 120, and 150 days. The BI values were increased with increasing storage time however, it was not significantly ( $p > 0.05$ ) different until 30 days of storage. It was found to be significantly ( $p < 0.05$ ) different for 90, 120, and 150 days of storage. The BI values were significantly ( $p < 0.05$ ) different for the main effect lactic acid. It was also significantly ( $p < 0.05$ ) different for time and the interaction effect time x lactic acid (Table 5.3). The BI values on 150 days of storage at 40°C were in the range 30.8 to 34.1 (AU). It was found that the BI values were comparatively higher for high temperature stored samples in comparison to room temperature samples. The results obtained were in accordance with those of Gómez-Narváez et al. (2019) who suggested that the BI values were higher at higher temperatures.



**Figure 5.8** Browning index values of milk permeate powders during storage at 40°C for 0, 30, 60, 90, 120, and 150 days.

Error bars are standard deviations from two independent trials (two lots).

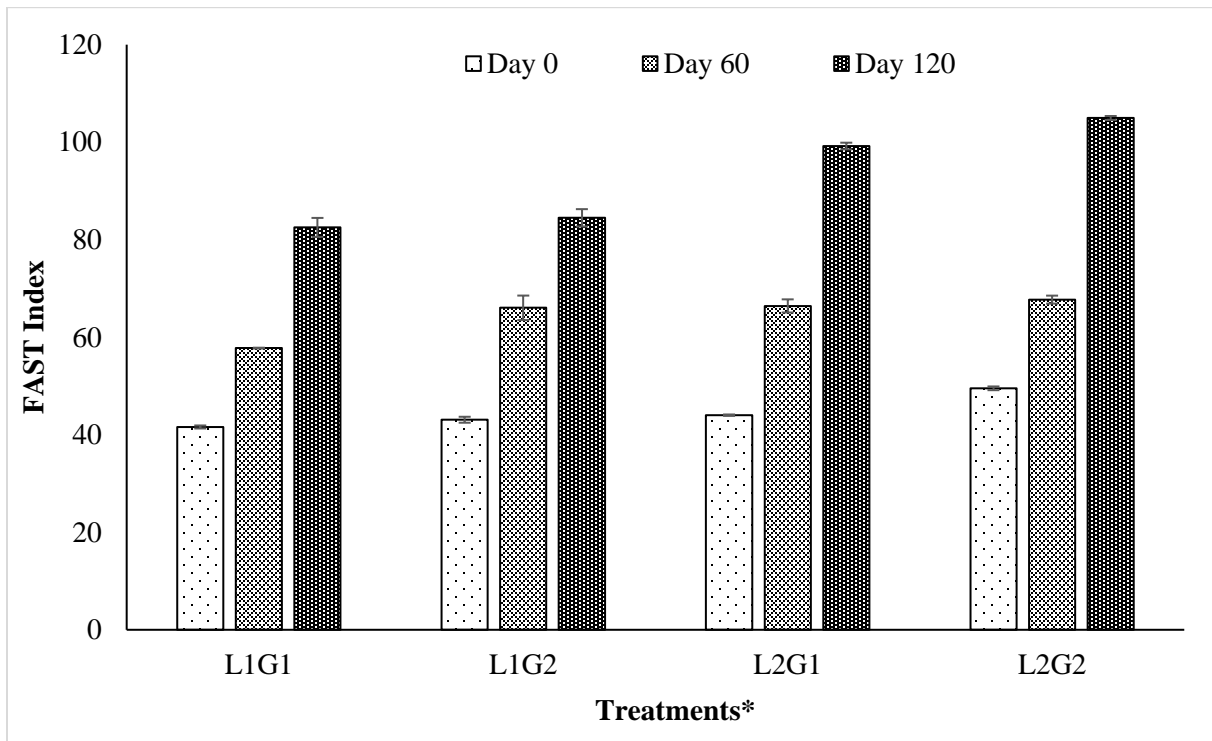
\*Treatments: L1G1 = low lactic acid low galactose; L1G2 = low lactic acid high galactose; L2G1 = high lactic acid low galactose; L2G2 = high lactic acid high galactose.

In general, it was observed that the BI values increased with increasing storage time for both temperatures 25°C and 40°C. The BI values of MP powders stored at 25°C were higher than those at 40°C. Similar results were observed by Dattatreya and Rankin (2004) who reported that the rate of browning was faster at higher temperatures (80 > 60 > 40°C) for sweet whey powder. Overall, it was observed that all the samples browned with time however, the rate of browning was observed to be higher at higher temperatures.

### **FAST Index**

The FI of MP powders right after production (fresh powders) were in the range of 41.59 to 49.52 which were found to be significantly ( $p < 0.05$ ) different. In fresh powders, the lowest FI value of 41.59 was for the sample containing low lactic acid (pH 6.26) low galactose (0%)

whereas the highest FI value of 49.52 was for the sample containing high lactic (pH 6) acid high galactose (0.3%).

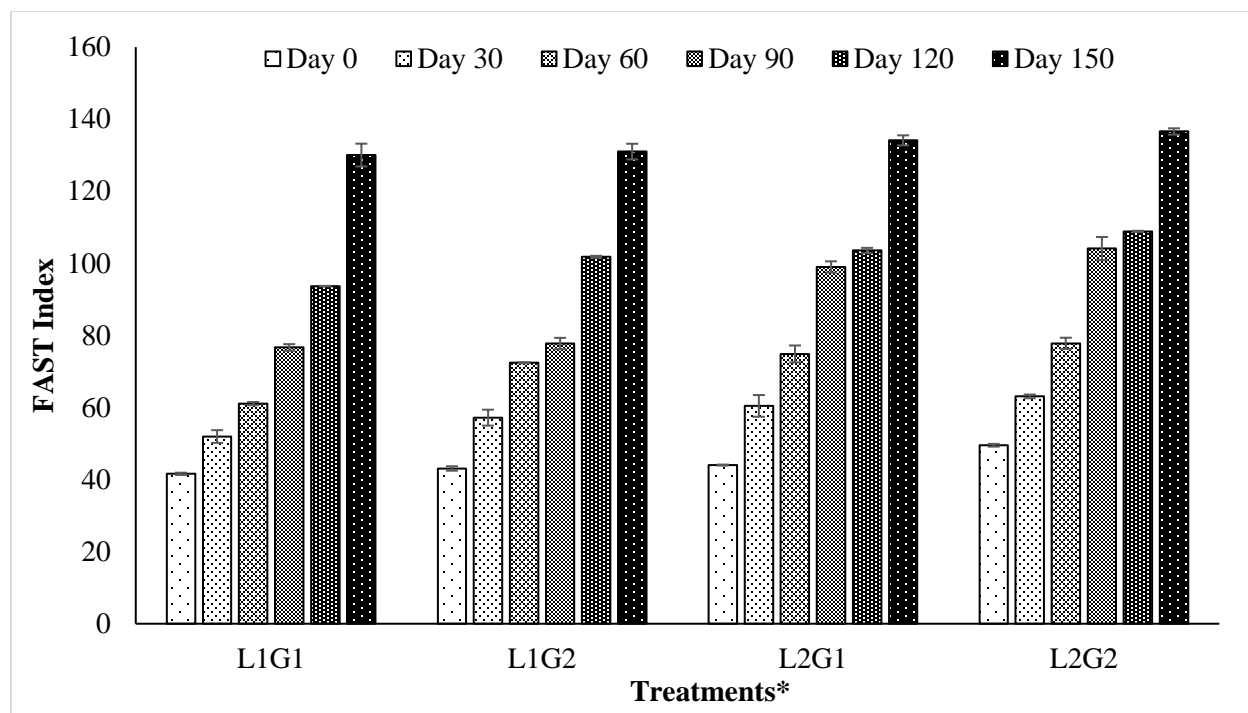


**Figure 5.9** FAST index values of milk permeate powders during storage at 25°C for 0, 60, and 120 days.

Error bars are standard deviations from two independent trials (two lots).

\*Treatments: L1G1 = low lactic acid low galactose; L1G2 = low lactic acid high galactose; L2G1 = high lactic acid low galactose; L2G2 = high lactic acid high galactose.

The FI values of MP powders during storage at 25°C for 0, 60, and 120 days is shown in Figure 5.9. The FI values increased significantly ( $p < 0.05$ ) with increasing storage time until 120 days of storage. The FI values were significantly ( $p < 0.05$ ) affected by lactic acid and galactose. On storage, it was found to be significantly ( $p < 0.05$ ) different for time and the interaction effects time x lactic acid, and time x lactic acid x galactose (Table 5.2). The FI values of MP powders on 120 days of storage at 25°C were observed to be in the range 82.52 to 104.96. The highest BI value of 104.58 on 120 days of storage was for the sample containing high lactic acid (pH 6) high galactose (0.3%).



**Figure 5.10** FAST index values of milk permeate powders during storage at 40°C for 0, 30, 60, 90, 120, and 150 days.

Error bars are standard deviations from two independent trials (two lots).

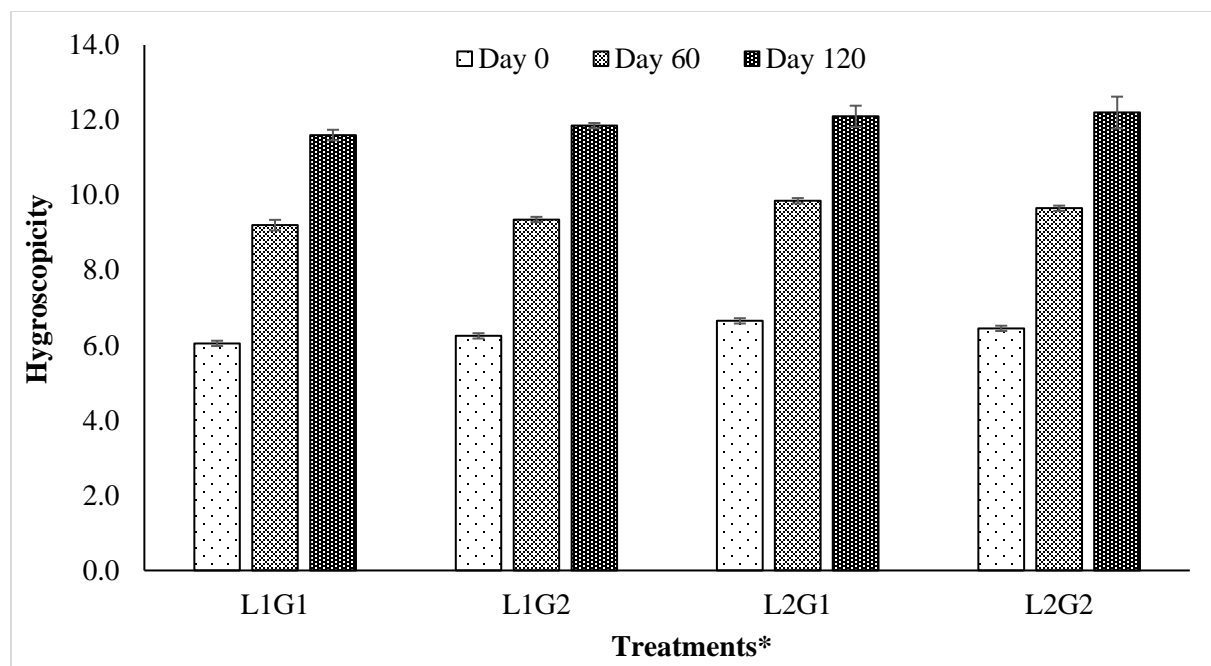
\*Treatments: L1G1 = low lactic acid low galactose; L1G2 = low lactic acid high galactose; L2G1 = high lactic acid low galactose; L2G2 = high lactic acid high galactose.

Figure 5.10 illustrates the FI values of MP powders during storage at 40°C for 0, 30, 60, 90, 120, and 150 days. The FI values increased significantly ( $p < 0.05$ ) with increasing storage time for all the storage days. The FI values were significantly ( $p < 0.05$ ) affected by lactic acid and galactose. On storage, it was found to be significantly ( $p < 0.05$ ) different for time and the interaction effects time x lactic acid, time x galactose and time x lactic acid x galactose (Table 5.3). The FI values of MP powders on 150 days of storage at 40°C were observed to be in the range 130.04 to 136.59. The highest FI value of 136.59 on 150 days of storage was for the sample high lactic acid high (pH 6) galactose (0.3%).

## Hygroscopicity

The ability of the powder to take up moisture from high humidity environment can be described as hygroscopicity. In dairy powders, this happens as a result of a strong interaction of water to lactose that is polar in nature (Haugaard et al., 1978; Slade and Levine, 1991). The hygroscopicity values of MP powders right after production were found to be in the range 6.1 to 6.7% which was found to be significantly ( $p < 0.05$ ) different only for the high lactic acid treated samples. The fresh powders right after production were observed as non-hygroscopic, free flowing powders. These powders can be regarded as non-hygroscopic according to Pisecky powder classification as the hygroscopicity values  $\leq 10\%$  when stored at 75% relative humidity (Pisecky, 1997; Schuck et al., 2004). Similarly, Schuck et al. (2004) reported a hygroscopicity value of 9.3% for ultrafiltered permeate powder which were higher than the MP powders in our study. Additionally, Shrestha et al. (2008) noted that minerals might affect the moisture sorption properties of MP powders, which in turn may lead to increased powder hygroscopicity.

The hygroscopicity values of MP powders during storage at 25°C for 0, 60, and 120 days is illustrated in Figure 5.11. It was found that the hygroscopicity values increased with increasing storage time as expected which was significantly ( $p < 0.05$ ) different for each storage day. The hygroscopicity values were significantly ( $p < 0.05$ ) affected by the main effect lactic acid and the interaction effect lactic acid x galactose. It was also found to be significantly ( $p < 0.05$ ) affected by time for the storage of MP powders at 25°C (Table 5.2). The hygroscopicity values for MP powders during storage at 25°C for 120 days were in the range 11.6 to 12.2% which reveals that these powders are in the category of slightly hygroscopic powders according to Pisecky powder classification (Pisecky, 1997; Schuck et al., 2012). The highest hygroscopicity values of 12.2% was for the sample containing high lactic acid (pH 6) high galactose (0.3%).



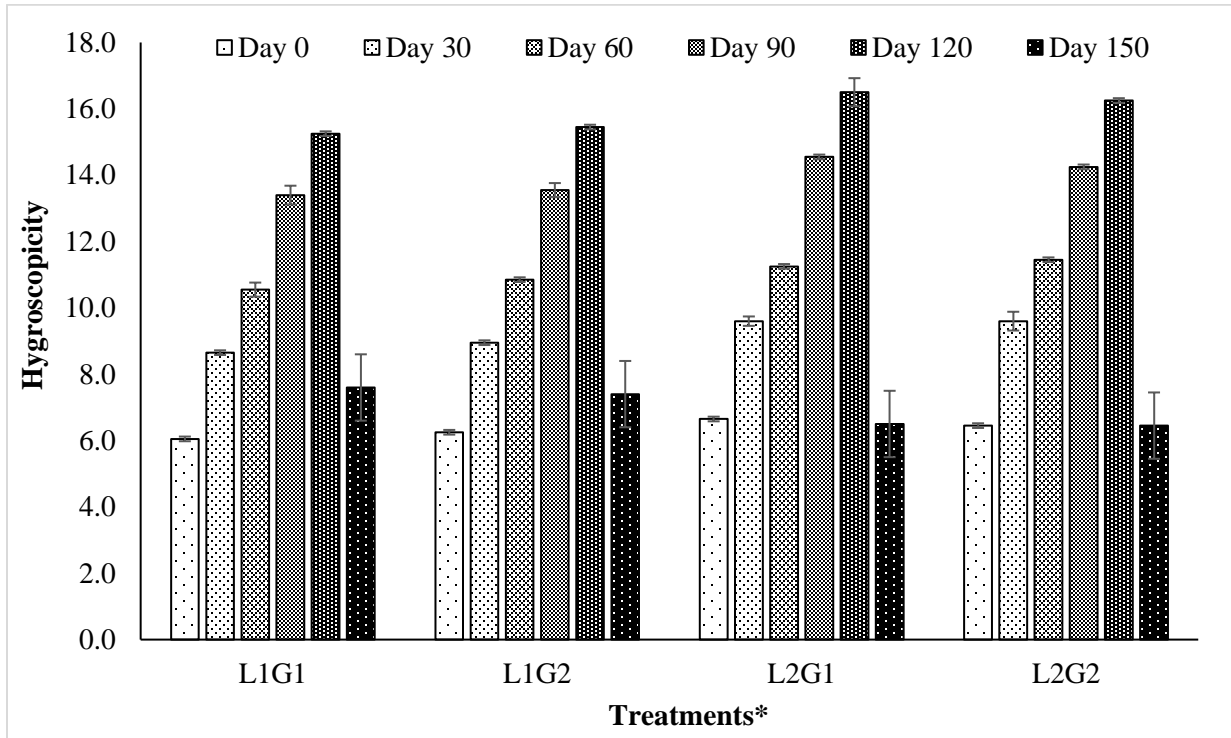
**Figure 5.11** Hygroscopicity values of milk permeate powders during storage at 25°C for 0, 60, and 120 days.

Error bars are standard deviations from two independent trials (two lots).

\*Treatments: L1G1 = low lactic acid low galactose; L1G2 = low lactic acid high galactose; L2G1 = high lactic acid low galactose; L2G2 = high lactic acid high galactose.

The hygroscopicity values of MP powders during storage at 40°C for 0, 30, 60, 90, 120, and 150 days is shown in Figure 5.12. Table 5.3 suggests that the hygroscopicity values were significantly different only with time on the storage of MP powders at 40°C. In general, the hygroscopicity values increased with increasing storage time until 120 days of storage but dipped suddenly for 150 days. The hygroscopicity values were not significantly ( $p > 0.05$ ) different with storage days. The sudden decrease in hygroscopicity values could be attributed to the rationale that the MP powders were caked completely by 150 days storage which meant that these powders could no longer absorb moisture after a certain level. The hygroscopicity values of MP powders during storage for 120 days of storage at 40°C were found to be in the range 15.3 to 16.5%. However, the hygroscopicity values for the MP powders on 150 days of storage at 40°C were found to be in the range 6.5 to 7.6%. The lowest value of 6.5% was for the samples with

high lactic acid treated samples. This could be because these powders could not absorb moisture anymore giving the lowest value which meant that these powders were the most caked among the others.



**Figure 5.12** Hygroscopicity values of milk permeate powders during storage at 40°C for 0, 30, 60, 90, 120, and 150 days.

Error bars are standard deviations from two independent trials (two lots).

\*Treatments: L1G1 = low lactic acid low galactose; L1G2 = low lactic acid high galactose; L2G1 = high lactic acid low galactose; L2G2 = high lactic acid high galactose.

### Caking

Figure 5.13 and Figure 5.14 shows the images of MP powder sample containing low lactic acid (pH 6.26) low galactose (0%) and high lactic acid (pH 6) high galactose (0.3%) on 0, 30, 60, 90, 120, and 150 days of storage at 40°C, respectively. Indeed, the main problems that occur in dairy powders are stickiness and caking mainly due to the presence of lactose (Huppertz and Gazi, 2016). The increase in the degree of caking with storage time were visually evident from both the figures. It is clear from these results that higher storage times lead to a greater risk



of caking. Previously, Carpin et. al. (2017) reported the presence of impurities in lactose powder was found to greatly enhance moisture sorption and caking. The MP powders right after production (day 0) were non-hygroscopic free flowing powders but after 30 days of storage, the powders were observed as slightly bigger particles than the fresh powder with particles coming together due to cohesion. A visual examination of the MP powders after 60 days of storage showed that they formed slightly larger lumps when compared to 30 days of storage. This suggests that the caking was progressing during storage. The caked lumps may be held together by strong or weak attachments. Although there is no published data on the relative size of the lumps or hardness of the caked powder which may represent as a benchmark of a severe caking problem, the MP powder started forming small agglomerated particles by 90 days of storage. Hartmann and Palzer, (2011) reported that the powder particles adhere to each other and start to get sticky often referred to as stickiness, that decreases the flowability of the powder in the primary stages of the caking process. This occurred as lactose crystals in the powders started absorbing moisture. Aguilera et al. (1995) proposed that caking follows bridging which occurs as a result of surface deformation and sticking at contact points between particles. However, by day 120 the powders started caking and can be seen as agglomerated caked particles or as lumps which can be broken if force is applied. Paterson and Bronlund (2009) reported capillary condensation mechanism leading to significant liquid bridging in crystalline lactose powder. Furthermore, at day 150 the powders were completely caked, and the caked particles were not able to be broken even if force was applied. This meant that the liquid bridges between them were converted to strong solid bridges which happened as a result of evaporation of water when the lactose recrystallized. Additionally, when inter-particle liquid bridges provide an

environment for dissolution of components and the resulting lactose crystallization transforms these interfaces into solid bridges, it leads to caking (Thomas et al., 2004)



**Figure 5.13** Images of milk permeate powder sample containing low lactic acid (pH 6.26) low galactose (0%) showing caking on different days of storage (A) Day 0 (B) Day 30 (C) Day 60 (D) Day 90 and (E) Day120 (F) Day150 at 40°C.

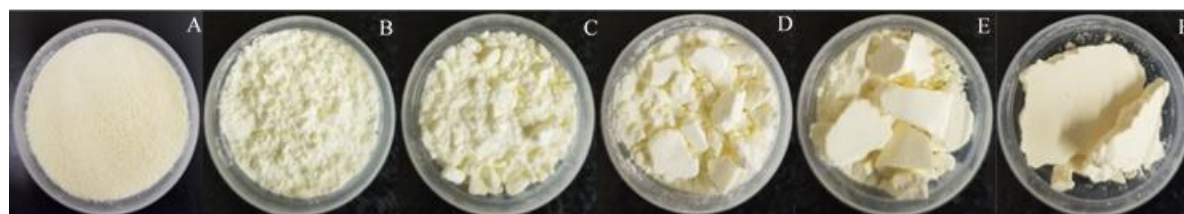
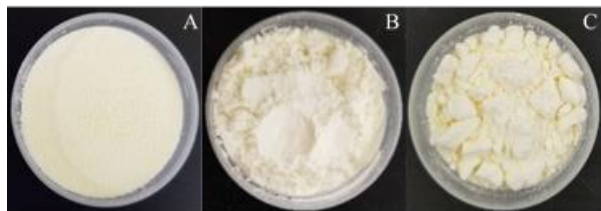
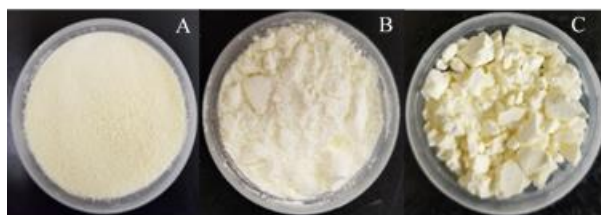


Figure 5.14 Images of milk permeate powder sample containing high lactic acid (pH 6) high galactose (0.3%) showing caking on different days of storage (A) Day 0 (B) Day 30 (C) Day 60 (D) Day 90 and (E) Day120 (F) Day150 at 40°C. Figure 5.15 and Figure 5.16 shows the images of MP powder samples containing low lactic acid (pH 6.26) low galactose (0%) and high lactic acid (pH 6) high galactose (0.3%) on 0, 60, and 120 days of storage at 25°C, respectively. As expected, the caking phenomenon was more prominent at higher temperatures (Kelly, 2009) in MP powders when compared to room temperature stored MP powders. It is evident from the figures that the caking phenomenon was much slower and not as high when compared to high temperature stored samples. Also, it could be observed that the caking was much lesser in the sample containing low lactic acid (pH 6.26) low galactose (0%) when compared to high lactic acid (pH 6) high galactose (0.3%). This could be due to the presence of lactic acid and galactose present in these samples. Lactic acid and galactose are hygroscopic in nature with a low Tg of -

60°C (Maltini et al., 1997) and 30°C (Roos, 1993), respectively, which makes the MP powders to absorb more moisture leading to caking.



**Figure 5.15** Images of milk permeate powder sample containing low lactic acid (pH 6.26) low galactose (0%) showing caking on different days of storage (A) Day 0 (B) Day 60 (C) Day120 at 25°C.

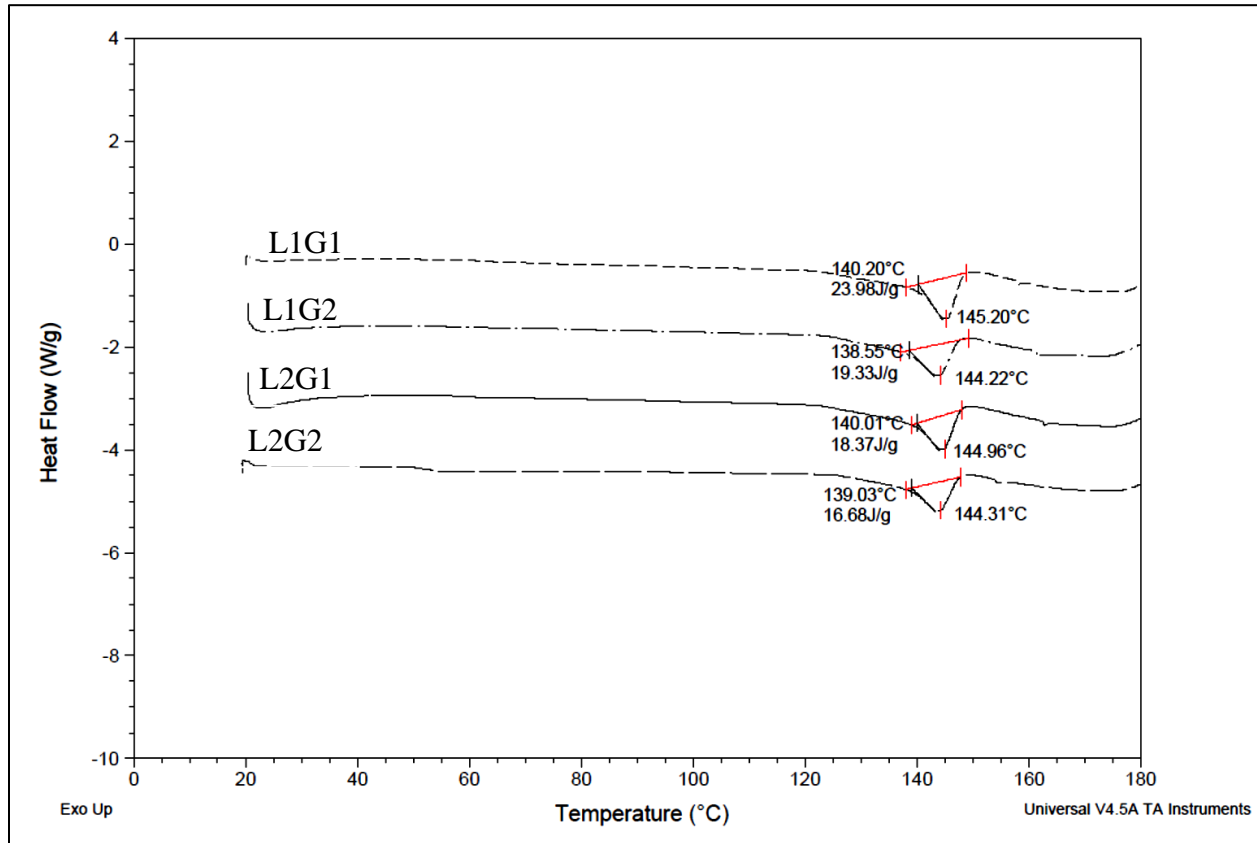


**Figure 5.16** Images of milk permeate powder sample containing high lactic acid (pH 6) high galactose (0.3%) showing caking on different days of storage (A) Day 0 (B) Day 60 (C) Day120 at 25°C.

### **Differential scanning calorimetry**

The DSC thermograms of MP powders right after production is shown in Figure 5.17. It can be observed that there is an endothermic peak at ~142°C for the MP powders which represent the loss of crystalline water. Similar results were reported by Gombas et al. (2002) for crystalline lactose which showed an endothermic peak at 144°C. This endothermic peak shows a peak shift for the treatments which could be due to the addition of lactic acid and galactose to the samples. Low lactic acid (pH 6.26) low galactose (0%) sample shows the highest enthalpy of 22.9 J/g whereas, the high lactic acid (pH 6) high galactose (0.3%) showed an enthalpy of 16.6 J/g. It has been reported that although lactose has a  $T_g$  of 101°C, its monosaccharide units glucose and galactose have a much lower  $T_g$  of 31°C and 30°C, respectively (Roos, 1993). Lactic acid is very hygroscopic and has a very low  $T_g$  of -60°C (Maltini et al., 1997; Chandrapala and

Vasiljevic, 2017). The rate of nonenzymatic browning is extremely low below the  $T_g$ , whereas, at temperatures greater than the  $T_g$ , this rate is higher (Karmas et al., 1992).



**Figure 5.17** Thermograms of milk permeate powders before storage.

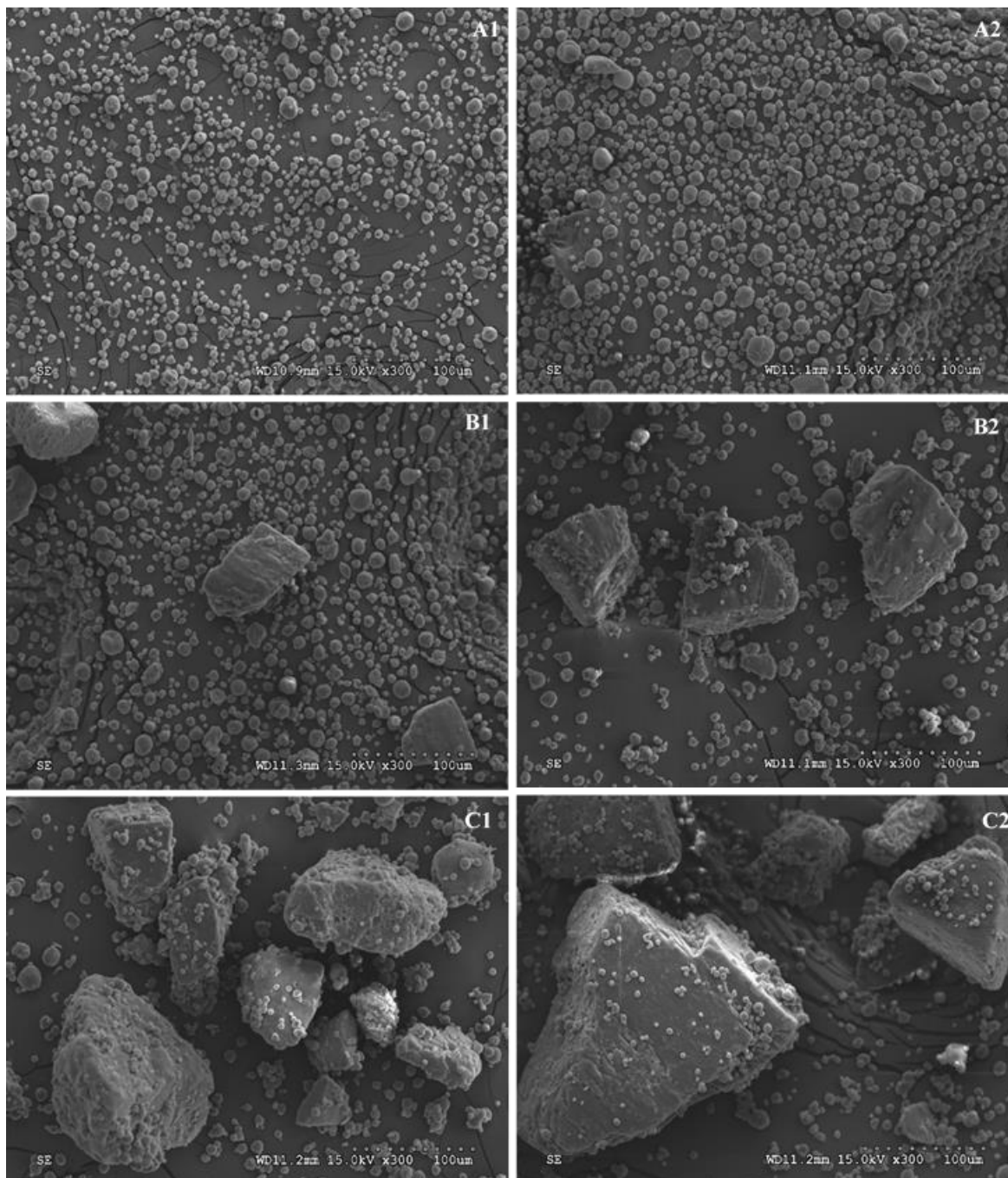
\*Treatments: L1G1 = low lactic acid low galactose; L1G2 = low lactic acid high galactose; L2G1 = high lactic acid low galactose; L2G2 = high lactic acid high galactose.

The obtained thermograms after storage also showed an endothermic peak around 143°C, which can be attributed to the removal of crystalline water (Wijayasinghe et al., 2015; Yazdanpanah and Langrish, 2011). An endothermic peak was observed at 192°C corresponding to  $\alpha$ -melt. Gombas et al. (2002) reported an endothermic peak at 213°C corresponding to  $\alpha$ -melt for 100% crystalline  $\alpha$ -lactose monohydrate. Chandrapala et al. (2016) also observed  $\alpha$ -melt at 218°C for pure lactose samples.

## Microstructure

The scanning electron micrographs ( $\times 300$ ) of spray dried MP powder sample containing low lactic acid (pH 6.26) low galactose (0%) and high lactic acid (pH 6.1) high galactose (0.3%) at day 0 (fresh powders), day 120 at 25°C, and day 120 at 40°C are shown in Figure 5.18. It can be observed that the powders right after production (fresh powders) were free flowing non-hygroscopic powders. These powders were less cohesive and did not touch each other. The fresh powders appeared as spherical/globular particles along with some sharp-edged particles. Some particles were found as less round with slight elongation. The fresh MP powders were observed as comparatively smooth particles. Žolnere and Ciproviča (2019) reported that sweet whey permeate powder was spherical in shape with a smooth surface. Similarly, sweet whey powder was noticed to be large spherical particles by Nishanthi et al. (2017). However, it was observed that the sample containing high lactic acid (pH 6) high galactose (0.3%) had larger particle size when compared to the sample low lactic acid (pH 6.26) low galactose (0%). Previous studies reported that the feed total solids content, droplet size, and drying temperature significantly affects the powder properties of the spray dried powder (Kim et al., 2009).

The SEM images of MP powders during storage at 25°C were found to have lesser caked particles in low lactic acid (pH 6.26) low galactose (0%) when compared to the high lactic acid (pH 6.1) high galactose (0.3%). Carpin et al. (2017) studied the presence of impurities in lactose powder by controlling the washing and purification steps and found that it greatly enhances moisture sorption and caking. Similar results were observed for the samples stored at 40°C. The possible explanation for this could be that lactic acid being very hygroscopic in nature tends to absorb more water thus leading to caking (Maltini et al., 1997; Shrestha et al., 2007).



**Figure 5.18** Scanning electron micrographs ( $\times 300$ ) of spray dried milk permeate powder samples (1) low lactic acid (pH 6.26) low galactose (0%) (2) high lactic acid (pH 6.1) high galactose (0.3%) at (A) Day 0 (fresh powders) (B) Day 120 at 25°C (C) Day 120 at 40°C.

The number of caked particles were much higher at 40°C in comparison to 25°C which implies that temperature has a significant impact on caking. This could be because due to the rise in temperature, there can be increased interparticle contact area leading to an increased number of contact points which results in the decreased distance between adjacent particles which could lead to caking as explained by (Mehos and Clement, 2008). Previous studies suggest caking was largely influenced by exposure to moisture, temperature variation, or attrition of particles. Authors have studied the effect of temperature on moisture sorption characteristics of lactose powder and temperature stimulated moisture migration in lactose bag (Bronlund and Paterson, 2004; Paterson and Bronlund, 2009).

## **Conclusion**

The study analyzed and differentiated the effect of the presence of impurities (lactic acid and galactose) on the storage stability of MP powders. Higher levels of lactic acid and galactose showed higher moisture sorption and a greater caking tendency when compared to lower levels of lactic acid and galactose. The higher lactose content and higher content of impurities associated with the particles increase the possibility of browning and caking. The findings of this study provide useful additional information on the effects of impurities on the physical behavior of lactose in MP powders. Improved understanding of these relationships is critical to the development of functional food systems. Such knowledge is also crucial for the development of effective process control systems for spray drying of lactose rich coproducts. Thus, this study evaluated the physiochemical and thermal properties of MP powders during storage and will be useful in optimizing its storage conditions.

## **References**

Aguilera, J., J. del Valle and M. Karel. 1995. Caking phenomena in amorphous food powders. Trends Food Sci. Technol. 6:149-155.

- AOAC International. 2002. Official methods of analysis. 17th ed. Association of Analytical Communities, Gaithersburg, MD.
- Bronlund, J. and T. Paterson. 2004. Moisture sorption isotherms for crystalline, amorphous and predominantly crystalline lactose powders. *Int. Dairy J.* 14:247-254.
- Carpin, M., H. Bertelsen, A. Dalberg, C. Roiland, J. Risbo, P. Schuck and R. Jeantet. 2017. Impurities enhance caking in lactose powder. *J. Food Eng.* 198:91-97.
- Chandrapala, J. and T. Vasiljevic. 2017. Properties of spray dried lactose powders influenced by presence of lactic acid and calcium. *J. Food Eng.* 198:63-71.
- Dattatreya, A., and S. A. Rankin. 2004. Browning of sweet whey powder as a function of pH. In Institute of Food Technologists (IFT) annual meeting, Las Vegas, NV, USA, Technical poster session: Dairy Food B. 17: 12-16.
- Dattatreya, A., and S. A. Rankin. 2006. Moderately acidic pH potentiates browning of sweet whey powder. *Int. Dairy J.* 16:822-828.
- Dattatreya, A., W. Lee and S. A. Rankin. 2010. Presence of galactose and glucose promotes browning of sweet whey powder. *J. Dairy Sci.* 93:2354-2357.
- Dec, B. and W. Chojnowski. 2006. Characteristics of acid whey powder partially demineralised by nanofiltration. *Pol. J Food Nutr. Sci.* 15:87-90.
- Fitzpatrick, J. J., T. Iqbal, C. Delaney, T. Twomey and M. K. Keogh. 2004. Effect of powder properties and storage conditions on the flowability of milk powders with different fat contents. *J. Food Eng.* 64:435-444.
- Gómez-Narváez, F., L. Pérez-Martínez, and J. Contreras-Calderón. 2019. Usefulness of some Maillard reaction indicators for monitoring the heat damage of whey powder under conditions applicable to spray drying. *Int. Dairy J.* 99:104553.
- Gonzales, A. P., G. B. Naranjo, G. E. Leiva and L. S. Malec. 2010. Maillard reaction kinetics in milk powder: Effect of water activity at mild temperatures. *Int. Dairy J.* 20:40-45.
- Hargrove, R. E., F. E. McDonough, D. E. Lacroix and J. A. Alford. 1976. Production and properties of deproteinized whey powders. *J. Dairy Sci.* 59:25-33.
- Hartmann, M. and S. Palzer. 2011. Caking of amorphous powders—Material aspects, modelling and applications. *Powder Technol.* 206:112-121.
- Haugaard, I. S., J. Krag, J. Pisecky and V. Westergaard. 1978. Analytical methods for dry milk powders. Niro Atomizer, Denmark.
- Hogan, S. A. and D. J. O'Callaghan. 2010. Influence of milk proteins on the development of lactose-induced stickiness in dairy powders. *Int. Dairy J.* 20:212-221.
- Hooi, R., D. M. Barbano, R.L. Bradley, D. Budde, M. Bulthaus, M. Chettiar, J. Lynch, R. Reddy, E. A. Arnold. 2004. Chemical and physical methods. In: Wehr, M.H., Frank, J.F. editors. Standard methods for the examination of dairy products. 17<sup>th</sup> edition. American Public Health Association. Washington, D.C. 363-532.



- Huppertz, T. and I. Gazi. 2016. Lactose in dairy ingredients: Effect on processing and storage stability. *J. Dairy Sci.* 99:6842-6851.
- Jaya, S. and H. Das. 2004. Effect of maltodextrin, glycerol monostearate and tricalcium phosphate on vacuum dried mango powder properties. *J. Food Eng.* 63:125-134.
- Jouppila, K. and Y. H. Roos. 1994. Water sorption and time-dependent phenomena of milk powders. *J. Dairy Sci.* 77:1798-1808.
- Juliano, P. and G. V. Barbosa-Canovas. 2010. Food powders flowability characterization: Theory, methods, and applications. *Annu. Rev. Food Sci. Technol.* 1:211-239.
- Kaanane, A. and T. P. Labuza. 1989. The maillard reaction in foods. *Prog. Clin. Biol. Res.* 304:301-327.
- Kedward, C. J., W. MacNaughtan and J. R. Mitchell. 2000. Crystallization kinetics of amorphous lactose as a function of moisture content using isothermal differential scanning calorimetry. *J. Food Sci.* 65:324-328.
- Kelly, P. M. 2009. Significance of Lactose in Milk Powders. Pages 80-97 in *Advanced Dairy Chemistry*. Vol. 3. P. McSweeney and P. F. Fox, ed. Springer, New York, NY.
- Kim, E. H., X. D. Chen and D. Pearce. 2009. Surface composition of industrial spray-dried milk powders. 2. effects of spray drying conditions on the surface composition. *J. Food Eng.* 94:169-181.
- Listiohadi, Y., J. Hourigan, R. Sleight, and R. Steele. 2005. An exploration of the caking of lactose in whey and skim milk powders. *Aust. J. Dairy Technol.* 60:207-213.
- Maltini, E., M. Anese and I. Shtylla. 1997. State diagrams of some organic acid-water systems of interest in food. *Cryo-Letters*.
- Maskan, M. 2001. Kinetics of colour change of kiwifruits during hot air and microwave drying. *J. Food Eng.* 48:169-175.
- Mehos, G., and S. Clement. 2008. Prevent caking and unintended agglomeration. *Chemical Engineering*. 115:55-61.
- Nishanthi, M., J. Chandrapala and T. Vasiljevic. 2017. Compositional and structural properties of whey proteins of sweet, acid and salty whey concentrates and their respective spray dried powders. *Int. Dairy J.* 74:49-56.
- Overend, W. G., A. R. Peacocke, and J. B. Smith. 1961. Reactions at position 1 of carbohydrates. part I. the polarographic reduction of carbohydrates. *J. Chem. Soc.* 3487-3497.
- Paterson, A. H., and J. E. Bronlund. 2009. The practical implications of temperature induced moisture migration in bulk lactose. *J. Food Eng.* 91:85-90.
- Pisecky, I. 1997. *Handbook of milk powder manufacture*. Copenhagen, Denmark: Niro A/S.
- Roos, Y. H. 1993. Melting and glass transitions weight carbohydrates of low molecular. *Carbohydr. Res.* 238:39-48.

- Schuck, P., A. Dolivet and R. Jeantet. 2012. *Analytical Methods for Food and Dairy Powders*. Wiley Online Library.
- Schuck, P., Dolivet, A. and Mejean, S. 2004. Lactose crystallization and hygroscopicity. *Advances in fractionation and separation: processes for novel dairy applications*. Bulletin of the IDF, 389: 10-15.
- Schuck, P., E. Blanchard, A. Dolivet, S. Méjean, E. Onillon, and R. Jeantet (2005). Water activity and glass transition in dairy ingredients. *Le Lait*. 85:295-304.
- Shrestha, A. K., T. Howes, B. P. Adhikari and B. R. Bhandari. 2007. Water sorption and glass transition properties of spray dried lactose hydrolysed skim milk powder. *LWT-Food Science and Technology*. 40:1593-1600.
- Shrestha, A. K., T. Howes, B. P. Adhikari and B. R. Bhandari. 2008. Spray drying of skim milk mixed with milk permeate: Effect on drying behavior, physicochemical properties, and storage stability of powder. *Drying Technol.* 26:239-247.
- Silalai, N. and Y. H. Roos. 2010. Roles of water and solids composition in the control of glass transition and stickiness of milk powders. *J. Food Sci.* 75: E285-E296.
- Slade, L., H. Levine and D. S. Reid. 1991. Beyond water activity: Recent advances based on an alternative approach to the assessment of food quality and safety. *Crit. Rev. Food Sci. Nutr.* 30:115-360.
- Stapelfeldt, H., B. R. Nielsen and L. H. Skibsted. 1997. Effect of heat treatment, water activity and storage temperature on the oxidative stability of whole milk powder. *Int. Dairy J.* 7:331-339.
- Thomas, M. E., J. Scher, S. Desobry-Banon and S. Desobry. 2004. Milk powders ageing: Effect on physical and functional properties. *Crit. Rev. Food Sci. Nutr.* 44:297-322.
- Thomsen, M. K., L. Jespersen, K. Sjøstrøm, J. Risbo and L. H. Skibsted. 2005. Water activity–temperature state diagram of amorphous lactose. *J. Agric. Food Chem.* 53:9182-9185.
- Žolnere, K. and I. Ciproviča. 2019. The study of physical properties of spray dried whey and milk permeates lactose. *Agronomy Research*. 17: 1501-1510.

## Chapter 6 - Conclusions

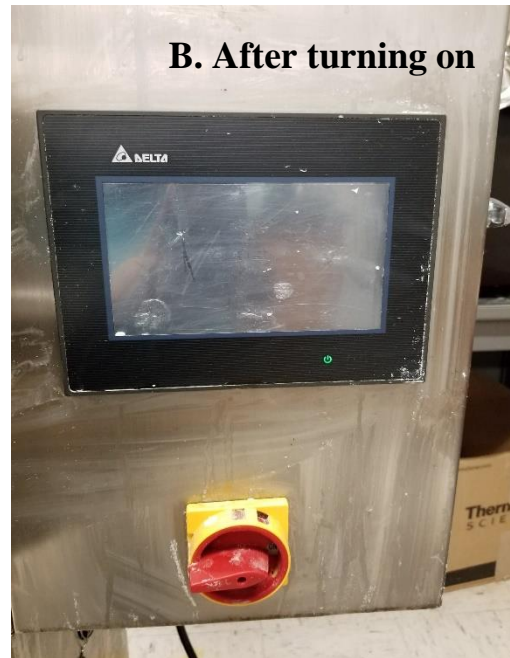
Increased manufacturing of cheese and high protein dairy ingredients continues to grow in retort to robust global demand. This increased production of cheese and high protein dairy ingredients has contributed to the rising production of lactose rich coproducts including whey permeate and milk permeate, creating attractive new opportunities for global food and beverage manufacturers to utilize these versatile ingredients in a wide range of applications. Deproteinized whey (DPW) is a coproduct of the production of whey protein concentrate and whey protein isolate. Good solubility and a pleasant dairy flavor make whey permeate formulator friendly. Milk permeate (MP) is a coproduct of the production of milk protein concentrate, milk protein isolate, and ultrafiltered milk manufacturing. Milk permeate has a similar composition to whey permeate, but because it is derived directly from milk and has fewer processing steps, its organoleptic profile may be different. Milk permeate is known for its clean, consistent flavor. Applications of permeate powders include baked goods, soups, sauces, confectionery, dry mixes, meats, dairy foods, beverages, and animal feed. Permeate powders provide cost-effective functional and flavor benefits to foods. As permeate powders have 76-85% lactose, the functionality of permeate is largely influenced by the lactose content. The ash contains calcium, phosphorus, and other valuable minerals, which will contribute to the overall mineral profile of a food product. However, there is a lack of fundamental understanding of the intrinsic and extrinsic factors affecting the DPW and MP powders, especially its stability during storage. The presence of lactose in these powders can adversely affect the powder properties which can cause undesirable changes such as, stickiness, and caking during processing/storage. Moreover, lactose being a reducing sugar could take part in Maillard reactions which could cause browning in these powders. In the last decade, limited research has been done in the examination of storage

stability in DPW and MP powders, halting improved understanding about reasons for their poor storage stability. The use of these powders is sometimes limited or reduced by their poor storage characteristics due to the formation of Maillard products and caking during processing and storage. However, the specific explanations for factors influencing the storage stability of DPW and MP powders are still evolving. Therefore, more insights and in-depth research are still needed to understand the influence of composition, processing, and storage conditions on DPW and MP powders. This study concluded the effect of different levels of lactic acid and galactose on the physical, chemical, and thermal properties of DPW and MP powders. Overall, DPW and MP powders tended to absorb moisture during storage. However, the powders with higher amounts of lactic acid and galactose were more sensitive to absorbing moisture, giving rise to lumping and caking problems. Overall, the findings from this study emphasize the importance of controlling the feed characteristics, production process, and storage conditions to reduce the Maillard reaction and limit caking in the final product and to enhance the storage stability of DPW and MP powders.

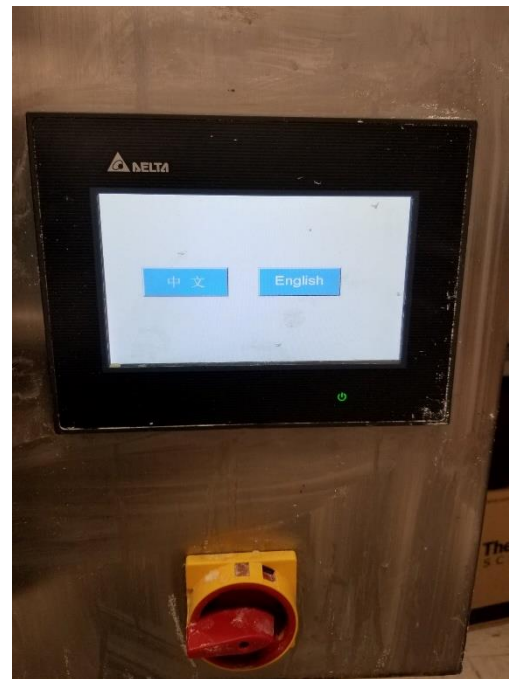
## Appendix A - Standard operating procedure for spray dryer

### Procedure for preparing the instrument before feeding

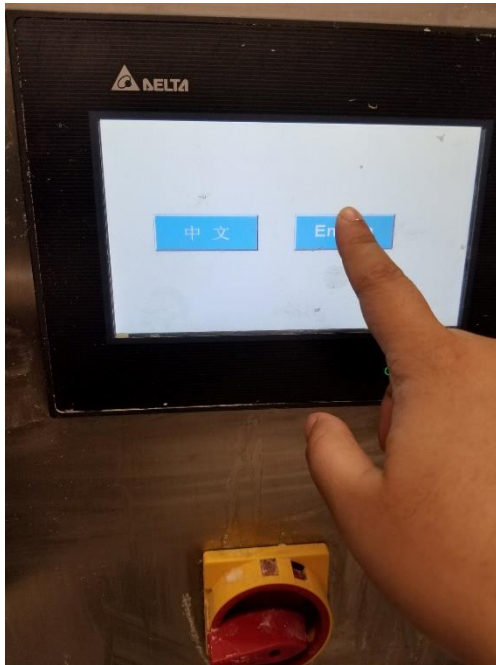
1. Turn on the spray dryer by turning the red switch to the clockwise direction.



2. This screen on the left will be displayed once the spray dryer is on and which will go automatically to the next where you should select the language.



- After selecting the language as English, the next screen will be displayed where you have to select the Lab scale spray dryer.

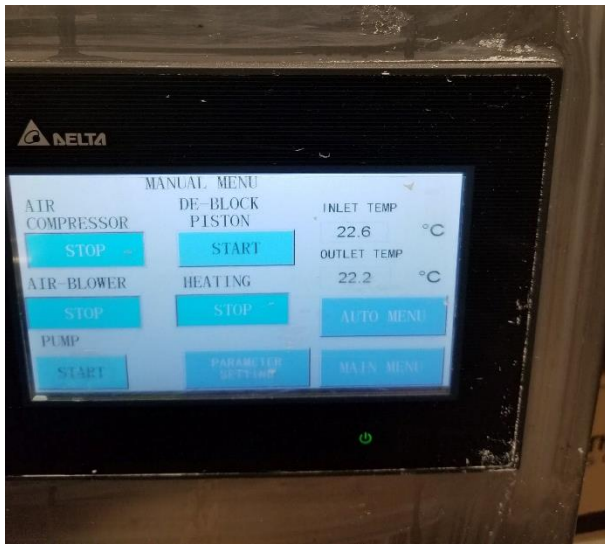


- Go to Parameter setting >> Page Down to get the screen on the right. Then set the inlet temperature to 135°C before starting the air compressor, air blower, and heating.

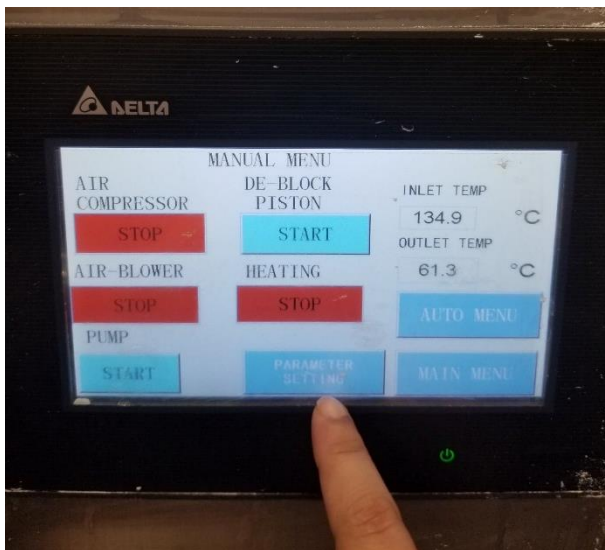


- This screen displayed below is showing the actual settings of a spray dryer. Usually, in a spray dryer, the inlet temperature is kept constant, although the inlet temperature you set depends on the type of material being dried. Start the Air compressor, Air blower, and the

Heating to start working with the spray dryer and wait for the inlet temperature to reach 135°C and after it stabilizes you can increase the set inlet temperature to that required for your spray drying experiment and bring it up to that temperature.



- To on the pump, you can press the start button for the pump. If you want to control the pump rate before starting the pump go to parameter setting >> pump (To set the pump rate, click on the pump and it will display a numerical tab (as shown on the right) where you can set the pump rate in rpm). When the outlet temperature reaches around 75°C you can start feeding water at a low pump rate (maybe around 5 rpm) and see if it is getting dried properly before feeding your actual sample. If you feel the water fed is being dried properly then stop the pump and you can wait until the outlet temperature reaches the temperature required for your feed to dry and then start feeding your sample at the pump rate desired.



### **Shut down instrument**

1. To shut down the instrument first stop the heating and the air compressor. Please note that you won't be able to stop the air blower until the temperature of the system reduces. So, you can stop the air blower after that.
2. Once you stop those, you can collect your powder from the powder collector.
3. After collecting the powder, you can switch off the red switch downwards which will off the whole system.
4. Please make sure to clean the inlet feed pipe by running hot water through it after disconnecting from the atomizer and putting it into a beaker so that the water doesn't go to the chamber.

### **Precautions**

1. Make sure that the parts of the spray dryer are dried properly before doing your experiment.
2. Please clean the part where the thermocouple is housed to make sure that it shows the right temperature.
3. Always make sure that the feed inlet pipe is clean without any residues from your previous run.
4. Please handle the instrument with care and make sure to clean it well after each use.
5. It is always better to gradually increase the pump rate rather than setting the desired one from the beginning so that you have better control over the outlet temperature.
6. Please make sure to clean the atomizer with care and make sure not to lose any small parts with it.

**Note:** If the particle size of the sample has to be adjusted then the pressure can be adjusted to do the same.



## Appendix B - SAS code for chapter 4 and 5

The water activity of the deproteinized powders during storage was analyzed using repeated measures using the SAS code given below.

```
data storage;
    input Lot LA$ GA$ Day0 Day30 Day60 Day90 Day150 Day210 Day270;
    datalines;
1      L      L      0.093 0.117 0.140 0.164 0.187 0.273 0.351
1      L      M      0.060 0.119 0.145 0.147 0.176 0.271 0.381
1      L      H      0.081 0.120 0.133 0.144 0.169 0.264 0.522
1      M      L      0.095 0.130 0.138 0.155 0.176 0.256 0.511
1      M      M      0.094 0.110 0.141 0.159 0.183 0.276 0.369
1      M      H      0.074 0.126 0.139 0.143 0.170 0.310 0.539
1      H      L      0.086 0.154 0.164 0.177 0.173 0.255 0.383
1      H      M      0.077 0.129 0.172 0.179 0.185 0.273 0.354
1      H      H      0.110 0.141 0.189 0.160 0.180 0.253 0.391
2      L      L      0.072 0.116 0.121 0.185 0.193 0.282 0.354
2      L      M      0.088 0.119 0.130 0.189 0.193 0.277 0.388
2      L      H      0.079 0.120 0.134 0.190 0.210 0.300 0.550
2      M      L      0.084 0.117 0.122 0.197 0.217 0.272 0.518
2      M      M      0.074 0.126 0.135 0.206 0.226 0.286 0.354
2      M      H      0.076 0.130 0.135 0.222 0.242 0.316 0.569
2      H      L      0.075 0.119 0.136 0.195 0.215 0.282 0.393
2      H      M      0.071 0.123 0.126 0.215 0.235 0.272 0.362
2      H      H      0.086 0.135 0.137 0.234 0.254 0.283 0.403
;
proc print data=storage;
run;
proc glm;
    class Lot LA GA;
    model Day0-Day270 = LA GA LA*GA / noint;
    repeated Time 7 (0 30 60 90 150 210 270) polynomial / summary printe;
run;
```

The water activity of the milk permeate powders during storage at 25°C was analyzed using repeated measures using the SAS code given below.

```
data storage;
    input Lot LA$ GA$ Day0 Day60 Day120;
    datalines;
1      L      L      0.095 0.127 0.179
1      L      H      0.103 0.128 0.183
1      H      L      0.108 0.136 0.203
1      H      H      0.111 0.139 0.210
2      L      L      0.096 0.127 0.183
2      L      H      0.105 0.130 0.186
2      H      L      0.115 0.140 0.206
;
proc print data=storage;
run;
proc glm;
    class Lot LA GA;
    model Day0-Day120 = LA GA LA*GA / noint;
    repeated Time 3 (0 60 120) polynomial / summary printe;
run;
```

The water activity of the milk permeate powders during storage at 40°C was analyzed using repeated measures using the SAS code given below.

```

data storage;
    input Lot LA$ GA$ Day0 Day30 Day60 Day90 Day120 Day150;
    datalines;
1     L     L     0.095 0.175 0.195 0.224 0.339 0.499
1     L     H     0.103 0.177 0.206 0.229 0.342 0.502
1     H     L     0.108 0.189 0.211 0.236 0.348 0.511
1     H     H     0.111 0.196 0.212 0.238 0.352 0.514
2     L     L     0.096 0.179 0.202 0.229 0.342 0.502
2     L     H     0.105 0.180 0.204 0.231 0.343 0.504
2     H     L     0.115 0.196 0.212 0.238 0.361 0.514
2     H     H     0.117 0.209 0.214 0.241 0.365 0.517
2     H     H     0.117 0.142 0.211
;
proc print data=storage;
run;
proc glm;
    class Lot LA GA;
    model Day0-Day150 = LA GA LA*GA / nouni;
    repeated Time 6 (0 30 60 90 120 150) polynomial / summary printe;
run;

```

The L\* of the deproteinized powders during storage was analyzed using repeated measures using the SAS code given below.

```

data storage;
    input Lot LA$ GA$ Day0 Day30 Day60 Day90 Day150 Day210 Day270;
    datalines;
1     L     L     92.43 92.04 91.93 91.49 90.25 89.84 85.05
1     L     M     92.11 91.83 91.57 91.35 91.10 89.23 85.37
1     L     H     91.83 91.31 91.00 89.80 89.72 87.10 78.27
1     M     L     92.17 91.66 91.28 90.56 89.95 88.86 82.28
1     M     M     92.75 92.65 92.63 92.29 91.06 88.73 83.47
1     M     H     92.43 92.40 92.38 92.02 91.39 87.56 83.16
1     H     L     92.24 92.04 91.93 91.49 90.85 88.54 83.71
1     H     M     92.20 92.40 92.00 92.76 91.48 88.75 83.46
1     H     H     92.11 91.95 91.83 92.67 90.98 88.89 84.28
2     L     L     91.85 91.17 90.29 90.96 90.25 88.80 84.85
2     L     M     91.67 90.36 89.65 90.87 90.61 88.51 84.80
2     L     H     92.39 92.16 92.64 91.24 90.25 87.86 77.60
2     M     L     92.21 91.46 91.54 89.79 89.43 88.59 81.79
2     M     M     92.87 92.71 92.58 91.41 90.48 88.33 82.63
2     M     H     92.78 92.61 91.84 90.93 90.59 87.78 83.10
2     H     L     92.48 91.89 92.09 91.36 90.89 88.37 82.31
2     H     M     92.94 92.87 92.76 91.65 90.84 88.54 83.19
2     H     H     92.90 92.66 92.63 91.94 91.05 88.72 83.85
;
proc print data=storage;
run;
proc glm;
    class Lot LA GA;
    model Day0-Day270 = LA GA LA*GA / nouni;
    repeated Time 7 (0 30 60 90 150 210 270) polynomial / summary printe;
run;

```

The L\* of the milk permeate powders during storage at 25°C was analyzed using repeated measures using the SAS code given below.

```

data storage;
    input Lot LA$ GA$ Day0 Day60 Day120;
    datalines;
1    L    L    93.58 91.40 89.73
1    L    H    93.32 91.28 89.64
1    H    L    92.93 91.09 89.33
1    H    H    92.74 91.00 89.11
2    L    L    94.30 92.74 90.35
2    L    H    94.32 92.56 90.16
2    H    L    93.57 92.37 89.90
2    H    H    93.47 92.11 88.78;
proc print data=storage;
run;
proc glm;
    class Lot LA GA;
    model Day0-Day120 = LA GA LA*GA / noint;
    repeated Time 3 (0 60 120) polynomial / summary printe;
run;

```

The L\* of the milk permeate powders during storage at 40°C was analyzed using repeated measures using the SAS code given below.

```

data storage;
    input Lot LA$ GA$ Day0 Day30 Day60 Day90 Day120 Day150;
    datalines;
1    L    L    93.58 92.43 90.82 88.62 79.95 73.62
1    L    H    93.32 92.20 90.65 88.20 79.78 73.53
1    H    L    92.93 91.62 90.16 87.38 78.50 72.24
1    H    H    92.74 91.53 90.05 87.19 78.00 72.09
2    L    L    94.30 93.65 91.68 89.74 80.72 73.96
2    L    H    94.32 93.53 91.47 89.40 80.50 73.77
2    H    L    93.57 93.12 90.22 89.02 79.87 73.14
2    H    H    93.47 92.98 90.25 88.84 79.22 73.03
;
proc print data=storage;
run;
proc glm;
    class Lot LA GA;
    model Day0-Day150 = LA GA LA*GA / noint;
    repeated Time 6 (0 30 60 90 120 150) polynomial / summary printe;
run;

```

The a\* of the deproteinized powders during storage was analyzed using repeated measures using the SAS code given below.

```

data storage;
    input Lot LA$ GA$ Day0 Day30 Day60 Day90 Day150 Day210 Day270;
    datalines;
1    L    L    -0.39 -0.55 -0.81 -0.86 -0.89 -0.73 1.54
1    L    M    -0.53 -0.78 -0.78 -0.90 -1.02 -1.06 0.08
1    L    H    -0.85 -0.79 -0.79 -0.79 -0.70 -0.82 4.00
1    M    L    -0.54 -0.74 -0.71 -0.71 -0.69 -0.85 1.95
1    M    M    -0.64 -0.84 -0.79 -0.94 -1.32 -1.06 1.87
1    M    H    -1.02 -1.19 -1.30 -1.35 -0.87 -0.26 0.97
1    H    L    -0.40 -0.57 -0.81 -0.86 -0.83 -0.88 1.93

```

```

1      H      M      -1.07 -1.14 -1.20 -0.94 -0.87 -1.02 0.64
1      H      H      -0.53 -0.74 -0.81 -0.78 -0.96 -0.94 0.41
2      L      L      -0.87 -1.17 -1.20 -1.33 -1.18 -0.81 1.30
2      L      M      -0.93 -1.15 -1.25 -1.35 -1.19 -1.01 1.29
2      L      H      -0.99 -1.18 -1.04 -1.39 -1.38 -1.07 4.05
2      M      L      -0.94 -0.95 -1.04 -1.31 -1.29 -1.09 1.99
2      M      M      -0.83 -1.14 -1.11 -1.34 -1.33 -1.24 1.93
2      M      H      -0.74 -1.07 -0.02 -1.43 -1.42 -0.78 1.30
2      H      L      -0.98 -1.25 -1.31 -1.47 -1.38 -1.09 2.14
2      H      M      -0.83 -1.02 -1.08 -1.30 -1.25 -1.15 0.98
2      H      H      -0.79 -0.99 -1.06 -1.40 -1.38 -1.07 1.03
;
proc print data=storage;
run;
proc glm;
class Lot LA GA;
model Day0-Day270 = LA GA LA*GA / nouni;
repeated Time 7 (0 30 60 90 150 210 270) polynomial / summary printe;
run;

```

The  $a^*$  of the milk permeate powders during storage at 25°C was analyzed using repeated measures using the SAS code given below.

```

data storage;
input Lot LA$ GA$ Day0 Day60 Day120;
datalines;
1      L      L      -2.67 -2.46 -1.95
1      L      H      -2.61 -2.41 -1.86
1      H      L      -2.57 -2.32 -1.76
1      H      H      -2.49 -2.28 -1.71
2      L      L      -2.74 -2.58 -2.11
2      L      H      -2.65 -2.52 -2.06
2      H      L      -2.45 -2.44 -1.97
2      H      H      -2.47 -2.40 -1.92
;
proc print data=storage;
run;
proc glm;
class Lot LA GA;
model Day0-Day120 = LA GA LA*GA / nouni;
repeated Time 3 (0 60 120) polynomial / summary printe;
run;

```

The  $a^*$  of the milk permeate powders during storage at 40°C was analyzed using repeated measures using the SAS code given below.

```

data storage;
input Lot LA$ GA$ Day0 Day30 Day60 Day90 Day120 Day150;
datalines;
1      L      L      -2.67 -2.62 -2.43 -2.24 -1.82 0.69
1      L      H      -2.61 -2.56 -2.41 -2.19 -1.71 0.71
1      H      L      -2.57 -2.49 -2.34 -1.88 -1.57 0.84
1      H      H      -2.49 -2.43 -2.34 -1.85 -1.32 0.86
2      L      L      -2.74 -2.66 -2.54 -2.47 -1.64 0.76
2      L      H      -2.65 -2.65 -2.51 -2.42 -1.58 0.79
2      H      L      -2.45 -2.47 -2.32 -1.77 -1.44 0.86
2      H      H      -2.47 -2.34 -2.23 -1.42 -1.39 0.93
;
proc print data=storage;

```

```

run;
proc glm;
  class Lot LA GA;
  model Day0-Day150 = LA GA LA*GA / nouni;
  repeated Time 6 (0 30 60 90 120 150) polynomial / summary printe;
run;

```

The b\* of the deproteinized powders during storage was analyzed using repeated measures using the SAS code given below.

```

data storage;
  input Lot LA$ GA$ Day0 Day30 Day60 Day90 Day150 Day210 Day270;
  datalines;
1 L L 14.71 14.60 14.62 14.60 15.82 15.90 25.31
1 L M 12.34 12.85 12.78 13.24 13.69 14.63 22.58
1 L H 14.24 15.29 17.03 17.90 18.17 18.30 29.16
1 M L 14.13 14.70 14.50 14.50 14.75 15.94 22.44
1 M M 12.45 12.96 13.23 13.36 14.52 15.34 20.47
1 M H 14.37 15.49 15.63 15.90 15.76 15.37 27.48
1 H L 14.72 14.63 14.65 14.60 15.14 15.31 23.29
1 H M 13.79 13.91 14.10 14.43 14.82 15.34 22.21
1 H H 13.25 13.45 13.59 14.63 14.94 15.45 23.56
2 L L 14.71 13.62 13.26 14.28 14.00 15.73 26.32
2 L M 12.34 13.17 13.84 13.76 14.12 15.84 24.30
2 L H 11.54 11.46 11.69 12.19 14.05 16.42 30.04
2 M L 13.76 13.85 13.79 13.77 16.81 17.88 24.41
2 M M 12.21 12.78 12.89 12.32 13.10 14.94 24.42
2 M H 12.47 12.99 13.54 13.39 15.01 18.01 27.71
2 H L 12.86 12.87 13.35 12.73 12.95 15.73 27.51
2 H M 11.82 11.75 12.36 11.93 12.45 14.88 24.33
2 H H 11.81 12.44 12.41 13.21 13.60 15.60 25.08
;
proc print data=storage;
run;
proc glm;
  class Lot LA GA;
  model Day0-Day270 = LA GA LA*GA / nouni;
  repeated Time 7 (0 30 60 90 150 210 270) polynomial / summary printe;
run;

```

The b\* of the milk permeate powders during storage at 25°C was analyzed using repeated measures using the SAS code given below.

```

data storage;
  input Lot LA$ GA$ Day0 Day60 Day120;
  datalines;
1 L L 13.46 14.68 15.06
1 L H 13.93 14.63 15.10
1 H L 14.12 14.56 15.18
1 H H 14.19 14.50 15.22
2 L L 14.52 15.25 16.56
2 L H 14.69 15.30 16.62
2 H L 14.28 15.41 16.79
2 H H 14.11 15.48 16.81
;
proc print data=storage;
run;
proc glm;

```

```

class Lot LA GA;
model Day0-Day120 = LA GA LA*GA / nouni;
repeated Time 3 (0 60 120) polynomial / summary printe;
run;

```

The b\* of the milk permeate powders during storage at 40°C was analyzed using repeated measures using the SAS code given below.

```

data storage;
input Lot LA$ GA$ Day0 Day30 Day60 Day90 Day120 Day150;
datalines;
1 L L 13.46 14.05 14.57 15.89 17.71 19.43
1 L H 13.93 14.16 14.79 15.88 18.29 19.55
1 H L 14.12 14.32 15.02 16.07 18.82 20.90
1 H H 14.19 14.57 15.08 16.12 19.15 21.37
2 L L 14.52 14.23 14.83 15.92 18.02 19.67
2 L H 14.69 14.17 14.88 15.93 18.36 19.72
2 H L 14.28 14.10 14.98 16.02 18.99 20.07
2 H H 14.11 14.02 15.03 16.06 19.08 20.45
;
proc print data=storage;
run;
proc glm;
class Lot LA GA;
model Day0-Day150 = LA GA LA*GA / nouni;
repeated Time 6 (0 30 60 90 120 150) polynomial / summary printe;
run;

```

The browning index of the deproteinized powders during storage was analyzed using repeated measures using the SAS code given below.

```

data storage;
input Lot LA$ GA$ Day0 Day30 Day60 Day90 Day150 Day210 Day270;
datalines;
1 L L 16.61 16.42 16.25 16.27 18.07 18.41 35.83
1 L M 13.61 14.08 14.04 14.55 15.06 16.59 30.04
1 L H 15.76 17.25 19.57 21.04 21.50 22.31 49.26
1 M L 15.81 16.46 16.30 16.44 16.92 18.58 32.89
1 M M 13.56 14.03 14.41 14.50 15.86 17.62 29.18
1 M H 15.67 16.94 17.03 17.41 17.77 18.63 40.03
1 H L 16.65 16.43 16.29 16.27 17.12 17.79 33.51
1 H M 14.95 15.01 15.26 15.75 16.54 17.66 30.77
1 H H 14.73 14.83 14.98 16.14 16.71 17.84 32.35
2 L L 16.33 14.84 14.51 15.57 16.77 18.35 37.37
2 L M 13.36 14.42 15.32 14.92 15.30 18.39 34.10
2 L H 12.22 12.01 12.33 12.86 18.43 19.28 51.59
2 M L 15.02 15.25 15.09 15.15 18.08 21.08 36.43
2 M M 13.09 13.56 13.74 13.03 15.02 17.03 35.96
2 M H 13.50 13.89 15.56 14.38 17.14 21.74 40.70
2 H L 13.82 13.71 14.22 13.44 15.49 18.21 41.62
2 H M 12.61 12.38 13.08 12.55 14.95 16.98 34.63
2 H H 12.64 13.27 13.19 14.00 15.68 17.98 35.59
;
proc print data=storage;
run;
proc glm;
class Lot LA GA;
model Day0-Day270 = LA GA LA*GA / nouni;

```

```

    repeated Time 7 (0 30 60 90 150 210 270) polynomial / summary printe;
run;

```

The browning index of the milk permeate powders during storage at 25°C was analyzed using repeated measures using the SAS code given below.

```

data storage;
    input Lot LA$ GA$ Day0 Day60 Day120;
    datalines;
1      L      L      13.01 15.06 16.29
1      L      H      13.67 15.06 16.44
1      H      L      14.01 15.09 16.69
1      H      H      14.19 15.05 16.84
2      L      L      14.13 15.43 17.99
2      L      H      14.41 15.58 18.15
2      H      L      14.19 15.82 18.52
2      H      H      13.99 15.99 18.85
;
proc print data=storage;
run;
proc glm;
    class Lot LA GA;
    model Day0-Day120 = LA GA LA*GA / nouni;
    repeated Time 3 (0 60 120) polynomial / summary printe;
run;

```

The browning index of the milk permeate powders during storage at 40°C was analyzed using repeated measures using the SAS code given below.

```

data storage;
    input Lot LA$ GA$ Day0 Day30 Day60 Day90 Day120 Day150;
    datalines;
1      L      L      13.01 13.96 15.05 17.37 22.70 30.62
1      L      H      13.67 14.18 15.38 17.50 23.77 30.90
1      H      L      14.01 14.53 15.83 18.20 25.20 34.19
1      H      H      14.19 14.92 15.92 18.35 26.18 35.19
2      L      L      14.13 13.95 15.14 16.97 23.10 30.95
2      L      H      14.41 13.90 15.26 17.10 23.75 31.17
2      H      L      14.19 14.03 15.78 17.87 25.11 32.19
2      H      H      13.99 14.06 15.92 18.26 25.54 33.03
;
proc print data=storage;
run;
proc glm;
    class Lot LA GA;
    model Day0-Day150 = LA GA LA*GA / nouni;
    repeated Time 6 (0 30 60 90 120 150) polynomial / summary printe;
run;

```

The hygroscopicity of the deproteinized powders during storage was analyzed using repeated measures using the SAS code given below.

```

data storage;
    input Lot LA$ GA$ Day0 Day30 Day60 Day90 Day150 Day210 Day270;
    datalines;
1      L      L      8.4    10.4 11.3 11.6 11.3 11.8 7.2
1      L      M      10.3 12.7 11.9 10.5 12.0 12.5 9.7
1      L      H      11.3 12.0 13.4 13.4 14.2 16.7 9.0

```

```

1      M      L      8.3    11.3   11.2   10.4   10.7   10.4   6.8
1      M      M      9.3    12.9   11.7   11.4   11.5   11.6   8.3
1      M      H      9.5    11.5   11.7   10.7   11.1   11.7   6.3
1      H      L      8.2    10.7   11.2   11.2   11.2   11.4   8.8
1      H      M      8.8    11.9   11.6   11.8   11.1   12.1   7.7
1      H      H      9.1    11.0   11.7   11.2   11.7   13.4   8.3
2      L      L      7.5    9.4    11.0   11.7   11.6   11.1   9.5
2      L      M      8.5    9.1    9.4    10.9   10.8   12.6   8.1
2      L      H      11.3   14.2   14.8   16.3   16.9   14.6   9.2
2      M      L      7.7    8.0    10.8   10.2   10.7   10.5   6.7
2      M      M      8.0    9.3    10.1   10.6   10.3   11.0   8.0
2      M      H      8.0    9.6    10.4   11.8   12.2   11.8   6.3
2      H      L      7.1    7.5    11.5   10.9   11.0   13.9   8.2
2      H      M      7.4    11.8   11.0   10.8   11.0   11.9   7.6
2      H      H      8.3    11.1   11.7   12.5   12.6   14.2   8.5
;
proc print data=storage;
run;
proc glm;
  class Lot LA GA;
  model Day0-Day270 = LA GA LA*GA / nouni;
  repeated Time 7 (0 30 60 90 150 210 270) polynomial / summary printe;
run;

```

The hygroscopicity of the milk permeate powders during storage at 25°C was analyzed using repeated measures using the SAS code given below.

```

data storage;
  input Lot LA$ GA$ Day0 Day60 Day120;
  datalines;
1      L      L      6.1    9.1    11.7
1      L      H      6.3    9.4    11.8
1      H      L      6.6    9.8    12.3
1      H      H      6.4    9.6    12.5
2      L      L      6.0    9.3    11.5
2      L      H      6.2    9.3    11.9
2      H      L      6.7    9.9    11.9
2      H      H      6.5    9.7    11.9
;
proc print data=storage;
run;
proc glm;
  class Lot LA GA;
  model Day0-Day120 = LA GA LA*GA / nouni;
  repeated Time 3 (0 60 120) polynomial / summary printe;
run;

```

The hygroscopicity of the milk permeate powders during storage at 40°C was analyzed using repeated measures using the SAS code given below.

```

data storage;
  input Lot LA$ GA$ Day0 Day30 Day60 Day90 Day120 Day150;
  datalines;
1      L      L      6.1    8.6    10.7   13.2   15.2   15.3
1      L      H      6.3    8.9    10.8   13.4   15.4   15.5
1      H      L      6.6    9.5    11.2   14.6   16.8   16.2
1      H      H      6.4    9.4    11.5   14.3   16.2   16.3
2      L      L      6.0    8.7    10.4   13.6   7.5    7.7

```



2	L	H	6.2	9.0	10.9	13.7	7.3	7.5
2	H	L	6.7	9.7	11.3	14.5	6.1	6.3
2	H	H	6.5	9.8	11.4	14.2	6.5	6.4

;

```
proc print data=storage;
```

```
run;
```

```
proc glm;
```

```
class Lot LA GA;
```

```
model Day0-Day150 = LA GA LA*GA / noint;
```

```
repeated Time 6 (0 30 60 90 120 150) polynomial / summary printe;
```

```
run;
```