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A Process to Produce Lactose Phosphate from Dairy Byproducts And Used as an Alternative to Emulsifying Salts In Processed Cheese Food Manufacture

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A PROCESS TO PRODUCE LACTOSE PHOSPHATE FROM DAIRY BY-PRODUCTS AND USED AS AN ALTERNATIVE TO EMULSIFYING SALTS IN PROCESSED CHEESE FOOD MANUFACTURE

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This dissertation is approved as a creditable and independent investigation by a candidate for the Doctor of Philosophy degree and is acceptable for meeting the dissertation requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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This dissertation is dedicated to my parents, wife, and all family and friends for their support and encouragement throughout my study.

Thank you for your support and encouragement

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ABBREVIATIONS

a*	Redness
a_w	Water activity
b*	Yellowness
CFR	Code of Federal Regulations
CIE	International Commission on Illumination
CN	Casein
DSP	Disodium phosphate
DSR	Dynamic stress rheometer
ES	Emulsifying salts
ESI	Electrospray ionization
FAC	Fibrous activated carbon
G'	Storage modulus
G"	Loss modulus
GAC	Granular activated carbon
HCl	Hydrochloric acid
Hz	Hertz

L*	Lightness
LaP1	Phosphorylation α-lactose monohydrate
LaP2	Phosphorylation milk permeate
LP	Lactose phosphate
LSD	Least significant difference
MB	Methylene blue
MCC	Micellar casein concentrate
MF	Microfiltration
mL	Milliliter
MP	Milk permeate
MPC	Milk protein concentrate
MPP	Milk permeate powder
MS	Mass spectrometry
NaOH	Sodium hydroxide
NC	Nature cheese
NFDM	Non-fat Dry Milk
PAC	Powder activated carbon

PC	Processed cheese
PCF	Processed cheese food
PCP	Processed cheese products
PCS	Processed cheese spread
Ph	Phenol
Pi	Inorganic phosphate
PVA	Rapid visco analyzer
psi	Pound-force per square inch
rpm	Revolutions per min
SMP	Skim milk powder
Tan δ	Loss modulus/storage modulus
TPA	Texture profile analysis
TS	Total solids
UF	Ultrafiltration
V	Volts
WMP	Whole milk powder
WPC	Whey protein concentrate

WPI	Whey protein isolate
α-LA	α-lactalbumin
αS_1-CN	αS_1 casein
αS_2-CN	αS_2 casein
β-CN	β-casein
β-LG	β-lactoglobulin
κ-CN	κ-casein
ΔE	Total color difference

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ABSTRACT

A PROCESS TO PRODUCE LACTOSE PHOSPHATE FROM DAIRY BY-PRODUCTS AND USED AS AN ALTERNATIVE TO EMULSIFYING SALTS IN PROCESSED CHEESE FOOD MANUFACTURE

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Lactose is the primary carbohydrate in most mammals' milk, commonly known as milk sugar. Milk permeate is a by-product of whey protein manufacturing through membrane technologies. It is cost-effective, available, and an excellent source of lactose. Sugar phosphorylation is a technique used to alter sugar's characteristics. It has numerous applications for developing dairy and food products. Lactose-6-phosphate (LP) is an organic compound that switches the hydrogen on lactose by monophosphate that has the potential to function as emulsifying salts (ES). ES, such as disodium phosphate (DSP) and trisodium citrate, have a critical effect on the emulsification characteristics of casein by sequestering the calcium from the calcium-paracaseinate phosphate complex in natural cheese during processed cheese (PC) manufacturing. PC is a dairy product manufactured by combining dairy and non-dairy components and heating the mixture with agitation to create a homogenous product with a long shelf life.

The first objective of this study was to develop a method to phosphorylate α-lactose (LaP1), and milk permeate powder (LaP2) at specific concentrations that include pH, temperature, and time. A mass spectrometry (MS) was used to define LP in both treatments. Two samples were applied as controls. Control 1 and control 2 were used α-

lactose monohydrate and milk permeate powder (MPP), respectively. The amount of lactose was lower in LaP1 (15.58%) and LaP2 (12.20%) compared to control 1 (69.32%) and control 2 (24.64%). However, the level of LP was increased in LaP1 (60.74%) and LaP2 (8.65%), which were 0.89 and 5.53% for control 1 and control 2, respectively. We conclude that lactose and milk permeate can be phosphorylated, and MS can be used to detect lactose and LP.

The objective of the second study was to remove the dark color of LaP1 and LaP2 solutions. During the phosphorylation process, the color of the solutions turns dark. Activated carbon has been utilized for decades to remove the dark color and improve the appearance of solutions. The usage of activated carbon has been expanded to include decolorization, gas separation and polluted air treatment, heavy metal recovery, and food processing with no hazard. This methodology is cheap method and environmentally friendly. The compositional characteristics of the solutions, such as pH, total solids (TS), and color parameters (L*- lightness, a*- redness, and b*- yellowness) were examined at different stages (seven stages) of washing the solutions. Both solutions' pH and TS decreased with increasing the number of washings with activated carbon. The L* of the initial solutions was lower than the final solutions. However, the a* and b* of the initial solutions were higher than the final solutions. The total color difference (ΔE) was calculated for both solutions. ΔE was decreased with increasing the number of washings with activated carbon in both solutions. The findings of this study indicate that activated carbon can be used to remove the dark color that results from the phosphorylation process.

The objective of the third study was to produce processed cheese food (PCF) with LaP1 (52% TS) instead of DSP. PCF is a dairy product prepared by blending dairy ingredients with non-dairy ingredients and heating the blend with agitation to produce a homogeneous product with an extended shelf-life. The ingredients in the PCF formulations were Cheddar cheese, butter, water, milk permeate powder, and LaP1 (at a ratio of 2.0, 2.4, 2.8, 3.2, 4.0, 5.0, and 6.0%) were formulated to contain 17.0% protein, 25.0% fat, 44.0% moisture, and 2.0% salt. The LP concentrations in LaP1 solutions were ranged between 0.63 to 1.9%. The PCF made with 2.0% DSP was also produced as a control. The PCF was analyzed for moisture, pH, end apparent cooked viscosity, hardness, melted diameter, and melting temperature. The moisture of PCF ranged from 42.3 to 44.0%, with a pH of 5.6 to 5.8. The end apparent cooked viscosity increased from 818.0 to 2060.0 cP as the level of LaP1 solution raised from 2.0 to 6.0%, while it was 660.0 cP in control. The hardness of PCF made with LaP1 elevated from 61.9 to 110.1 g as the level of LaP1 increased; however, it was 85.6 g in control. The melted diameter decreased from 43 mm in control to 29 mm in 6% LaP1, while the melting temperature of PCF increased from 37.7°C in control to 59.0°C in 6% LaP1. We conclude that LaP1 can be utilized as a substitute for DSP in PCF manufacture.

The objective of the final study was to produce PCF using LaP2 (70% TS). The amount of LP was 0.48%. The ingredients in the PCF formulations were Cheddar cheese, butter, water, MPP, and LaP2 (8.0%). Those ingredients were formulated to contain 17.0% protein, 25.0% fat, 43.0% moisture, and 2.0% salt. PCF with 2.5% DSP was also produced as a control. The experiment was repeated 5 times using five different batches of LaP2 solutions. The moisture of PCF ranged from 42.61 and 43.09%. The pH was 5.81

for PCF made with LaP2; however, it was 5.74 in control. The cooked viscosity of LaP2 was 2032.0 cP, while it was 1378.0 cP in control. The hardness of PCF made with LaP2 was 154.5 g and 91.6 g in control. The melted diameter decreased from 41.0 mm in control to 34.0 mm in LaP2, while the melting temperature of PCF increased from 43.2°C in control to 46.5°C in LaP2. We conclude that LaP2 can be utilized as a substitute for DSP in PCF manufacture.

Keywords: Processed cheese food; Lactose-6-phosphate; Emulsifying salts; Milk permeate; Functional properties; Phosphorylation; Activated carbon

CHAPTER I: INTRODUCTION AND OBJECTIVES

1. Significance of the research

Processed cheese (PC) is a stable oil-in-water emulsion. It is made by blending natural cheese (NC) with emulsifying salts (ES) and other dairy and non-dairy ingredients, then heating and mixing to create a homogenous product with a long shelf-life. Pasteurized processed cheese (fat ≥ 30%, moisture ≤ 40%, and pH ≥ 5.3), pasteurized processed cheese food (fat ≥ 23%, NC ≥ 51%, moisture ≤ 44%, and pH ≥ 5.0), and pasteurized processed cheese spread (fat ≥ 20%, moisture= 44-60%, and pH ≥ 4.0) are PC categories that differ depending on moisture, fat, pH, and NC contents (Kapoor and Metzger, 2008). ES has a critical effect on the emulsification characteristics of casein (CN) by sequestering the calcium from the calcium-paracaseinate phosphate complex in NC. Mono-, di-, trisodium phosphates, sodium hexametaphosphate, sodium acid pyrophosphate, tetrasodium pyrophosphate, sodium aluminum phosphate, sodium citrate, potassium citrate, dipotassium phosphate, calcium citrate, sodium potassium tartrate, and sodium tartrate are examples of ES used in the PC.

Sodium is essential for most ES currently used in PC. When administered at the recommended daily intake concentration, these minerals play critical functions in human metabolism. However, excessive ingestion of any element may negatively impact and produce health concerns. For example, the total sodium in PC made using 2.5% disodium phosphate (DSP) is 1876.5 mg/100g. High sodium intake in human nutrition is a risk factor for various disorders, notably causing high blood pressure and heart disease (Wang and Labarthe, 2011).

On the other hand, phosphorus is an essential element in most ES used in PC. The total phosphorus in PC made using 2.5% DSP is 831.5 mg/100g. High phosphorus intake in human nutrition is a risk factor for hyperphosphatemia and has been associated with high phosphorus ingestion or renal impairment (Kheadr et al., 2021). The word "hyperphosphatemia" refers to high phosphate concentrations in the blood, which may result in the formation of calcium deposits in soft tissue. Additionally, the amount of lactose in PC formulation should be less than 17% lactose% water to prevent the crystallization process in PC.

Lactose phosphate (LP) is a compound naturally present in milk and milk products with low percentages (Lifran et al., 2007; Thum et al., 2015). LP was discovered for the first time in bovine milk (Barba and Caputto, 1965) and was recently discovered in caprine milk (Albrecht et al., 2014). LP is an organic compound that switches the hydrogen on lactose by monophosphate. Lactose and LP are structurally identical. The bulk of LP molecules has a phosphate group attached to the lactose galactose moiety. Around 90% of LP in pharmaceutical-grade lactose is connected to galactose; 10% of LP is attached to glucose (Breg et al., 1988).

Little was known about LP, its background, and its influence on lactose and PC functionality. Obtaining the right LP combination, finding pure lactose devoid of LP and other contaminants, directly analyzing LP, and studying the emulsifying properties of LP were the difficulties that led to the limitations of the studies on LP. There are no published data about LP's impact on PC. Similarly, the absence of knowledge of this compound's origin is notable. LP is a lactose-derived chemical molecule with the potential to function as an ES. Visser (1988) found that the crystal growth of

pharmaceutical-grade lactose was inhibited with the presence of LP. The crystallization of lactose in PCF led to adding a certain amount in the formulation. Using LP in PCF as ES could help increase lactose in PCF formulation. However, this could affect the functionality of PCF when lactose% water is higher than 17%. Moreover, it could reduce the amount of sodium and phosphorus in PCF that came from ES itself.

This dissertation hypothesizes that the utilization of LP could be proper from dairy by-products and could be used as an alternative to ES for PCF manufacture. The main goal of this dissertation was to create a method for producing PCF using LP made from dairy by-products and study the functional properties of PCF. In this work, LP was made in two ways: 1) Using α-lactose monohydrate and sodium cyclo-triphosphate, and 2) Using milk permeate powder (MPP) and DSP. The specific objectives were to:

- Synthesis of LP using α-lactose monohydrate and sodium cyclo-triphosphate stirred for three days at room temperature and pH of 12 (chapter 3).

- Estimation and identification of lactose and LP made by lactose and sodium cyclo-triphosphate (LaP1) using Mass spectrometry (MS) (chapter 3).

- Synthesis of LP utilizing MPP and DSP (LaP2) stirred for three days at room temperature and pH 12 in a ratio of 28.32% and 1.41%, respectively (chapter 3).

- Estimation and identification of lactose and LP made by MPP and DSP using MS (chapter 3).

- Assess the total solids (TS) and pH for LaP1 and LaP2 (chapter 3).

- Decolorize LaP1 and LaP2 solutions with activated carbon and concentration of the solutions using the evaporation technique (chapter 4).

- Assess the total solids and pH for LaP1 and LaP2 at different stages of washing with activated carbon (chapter 4).

Assess differences in color recorded in the CIE L*a*b* scale in terms of lightness (L*) and color (a* – redness, b* – yellowness) for LaP1 and LaP2 solutions at different stages of washing with activated carbon (chapter 4).

Assess the total color difference (ΔE) for LaP1 and LaP2 solutions at different stages of washing with activated carbon (chapter 4).

- Formulation PCF to obtain 17% protein, 25% fat, 44% moisture, and 2% salt using Cheddar cheese, butter, water, and MPP (chapter 5).

- Assess the emulsifying of LP using seven concentrations of LaP1 solutions (2, 2.4, 2.8, 3.2, 4, 5, and 6%), compared to PCF made with 2% DSP as control (chapter 5).

- Assess PCF's functionalities that include TS, pH, cooked viscosity, hardness, melted diameter, and melting temperature (chapter 5).

- Formulate PCF using Cheddar cheese, butter, water, and MMP to provide 17% protein, 25% fat, 43% moisture, and 2% salt (chapter 6).

- Compare the emulsification of 8% of LaP2 solutions to PCF manufactured with 2.5% DSP as the control (chapter 6).

- Assess PCF's functionalities that include TS, pH, cooked viscosity, hardness, melted diameter, and melting temperature (chapter 6).

The collected results provide essential information on LP that can help new possibilities for product development of milk-based and food goods. LP can be produced using either α-lactose or MPP and utilized as a substitute for DSP in PCF manufacture. LP influences the functionalities of PCF. LP could be used to decrease sodium and phosphorus in PCF.

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CHAPTER II: REVIEW OF LITERATURE

2.1 Processed cheese

2.1.1 Definition and types of processed cheese (PC)

The primary distinction between natural and processed cheese (PC) is that PC is not made directly from milk (Chen and Liu, 2012). PC is primarily made up of natural cheese (NC). PC has been known for more than a century around the world. The purposes of the patent were to increase the shelf-life and save the quality of hard and semi-hard cheese that could be exported worldwide. The demand for PC has increased due to the long-term shelf-life, which is the case of the high temperature of processing and packaging.

Moreover, the varieties of PC and multitude of compositions have assisted in spreading it globally by meeting different demands from consumers. PC is made by blending dairy and non-dairy products at various temperatures, stirring, and pH with the presence of emulsifying salts (ES) (Kapoor and Metzger, 2008). The dairy products include butter, NC, cream, anhydrous milk fat, curd, milk powder, whey powder, and caseinates. Non-dairy products contain coloring, hydrocolloids, vegetable oil, salt, and ES. Thus, the PC can be defined depending on the moisture and fat contents, pH level, quality, and the number of ingredients used in the PC. PC is divided into three categories by the Code of Federal Regulations (CFR): pasteurized processed cheese (PC), pasteurized processed cheese food (PCF), and pasteurized processed cheese spread (PCS)

(Code of Federal Regulations, 2003). On the other hand, pasteurized processed cheese product (PCP) is an undefined category that resembles other categories but may contain ingredients not permitted in other categories (Kapoor and Metzger, 2008).

There are four standards of identity categories of PC:

2.1.1.1 Processed cheese

PC is made by mixing hard and semi-hard NC with salt and ES and cooked at a temperature between 71–80°C for 5 to 8 min (Kosikowski and Mistry, 1977). According to the Food and Drug Administration (FDA), PC is defined as a product prepared from NC (70 to 80%), ES (3%), no more than 40% moisture, and no less than 30% fat content in the finished product guidelines. It contains dairy (anhydrous milk fat, cream) and non-dairy ingredients (acid, water, salt, color, and emulsifying agent). The pH of the final product should be no less than 5.3 (5.6 to 5.8) (Kapoor and Metzger, 2008).

2.1.1.2 Processed cheese food

PCF is made on the same basis as PC; however, it must have higher moisture (≤ 44%), less fat content (≥ 23%), and 51% of NC in the final product. The pH of the product ranges from 5.2 to 5.6 (Kosikowski and Mistry, 1977; Meyer, 1973; Thomas, 1973). The cooking temperature is ranged from 79 to 85°C. It contains dairy ingredients (whey, milk permeate, anhydrous milk fat, cream, milk, skim milk), organic acids (lactic acid and citric), and other flavorful food ingredients.

2.1.1.3 Processed cheese spread

PCS has the same basis as PC, but it must have higher moisture (44-60%) and lower fat content (≥ 20%) compared to other categories of PC (Kosikowski and Mistry, 1977). The cooking temperature is ranged from 88 to 91°C. It contains gums and sweetening agents in the product, and the pH of the final product must be higher than 4. Dips, sauces, and spreads may benefit PCS (Hammam, 2019).

2.1.1.4 Processed cheese product

PCP is an undefined category, which means it can contain any ingredients in formulations. It could be similar compositions to the above, but it has different ingredients.

2.1.2 History of processed cheese

PC has been around since the early twentieth century (Meyer, 1973). In 1895, PC was first manufactured without ES, but it was not successful until using citrates and phosphates as ES when industrial PC manufacturing became practical. Fondue, a Swiss national dish in which cheese is heated (melted) in wine containing tartrate, which works as an emulsifier, may have inspired the idea (Carić and Kaláb, 1999). In 1911, Swiss cheese was melted with sodium citrate as the ES to make a smooth, homogeneous product, invented by Walter Gerber and Fritz Stettler of Gerber & Co (Kapoor and Metzger, 2008). In 1912, a Swiss patent for citrates was granted, and manufacturing began across Europe. Kraft, who employed citrates and orthophosphate as ES in cheese production, created PC independently in the United States later (1917).

2.1.3 Principle of making processed cheese

When producing PC, the following principles are followed: adjusting pH, calcium sequestration, water binding, and emulsification using ES, followed by mixing, heating, and cooling the final product (Henning et al., 2006). The optimum combination of ES often increases the pH of cheese (generally from 5.0–5.5 in NC to 5.6–5.9 in PC) because of their strong buffering capacity. The increased pH results in a more reactive paracaseinate/caseinate confirmation with a more significant potential for water binding. When the pH is often substantially lower, the calcium bound to casein is significant. The chemistry of PC relay on the casein and ES and how they react during manufacturing. Calcium and phosphate play a role in misrule casein structure (cross-linking agent) that helps hold all casein fractions together. ES is typically phosphate or citrate come with sodium, and when dissolved in water, they will release sodium ion and have a negative charge of phosphate or citrate that will react with Ca (take the Ca away from casein) that, will cause the casein to disaggregate and act as individual molecular. Casein has a hydrophobic part that will catch and interact with fat, and a hydrophilic catch interacts with water with heating and mixing during the PC process, then cools to form a gel. The reactivated casein then binds the free water and emulsifies the free fat produced during processing, playing a critical role in creating a stable PC. These modifications are verified by the following: 1) the significant increase in water-soluble protein content after processing (from 5% to 20% in NC to 60% to 80% in PC); 2) the high concentrations of insoluble calcium (60 to 80% of total calcium) and phosphate in PC across a variety of levels and kinds of ES utilized; 3) the decrease in fat globule size during processing (Guinee, 2009). The amount and kind of ES used and the production circumstances and

qualities of NCs all impact PC quality (Kapoor and Metzger, 2008). Calcium phosphate para-caseinate or calcium-casein-phosphate is changed from an insoluble to a soluble state during PC production by ES in the presence of heating and shear action while mixing the constituents of PC. Consequently, the PC becomes more physiochemically stable by binding water and emulsifying fat (Guinee, 2011).

2.1.4 The effect of ingredients on the functional properties of processed cheese

2.1.4.1 Intact casein

Spherical-shaped colloid particles (about 40–300 nm in diameter) called casein micelles are found in milk, accounting for around 80 to 100% of the total protein in milk and being the primary structural protein in acid-induced milk gels (Fox and Brodkorb, 2008). CN is heterogeneous, consisting of four major types: αs1, αs2, β, and κ -CNs, that are present in the ratio 4:1:4:1. The kind and quantity of protein significantly affect PC properties (Salunke, 2013). CN is a critical structural and emulsifying protein in cheese. CN or caseinates improve the uniformity of PC formulations (Shimp, 1985). The most significant element in PC formulations is intact casein, chosen based on the cheese's kind, maturity, flavor, consistency, texture, and pH (Zehren and Nusbaum, 2000). The quantity of intact casein in NC also influences the PC characteristics (Brickley et al., 2007; Kapoor and Metzger, 2008; Templeton and Sommer, 1932; Zehren and Nusbaum, 2000). Intact casein is known as non-hydrolyzed casein, abundant in fresh cheese but diminishes during ripening due to proteolysis (Purna et al., 2006). This happens when the enzymes and residual starter or non-starter lactic acid bacteria in NC hydrolyze the proteins in the cheese into peptides, lowering the quantity of CN remaining in its intact (unhydrolyzed)

form (Kapoor and Metzger, 2008). The amount of intact casein in any variety is regulated by a range of parameters (e.g., age, ripening circumstances, milk pretreatments, composition, coagulant/culture type, and the addition of exogenous enzymes). Thus, the determination of intact casein content (e.g., by measuring the amount of protein insoluble in water at pH 4.6, gel electrophoresis/densitometry, and reversed-phase HPLC) is increasingly being used as a quality control tool in the selection of NC and determining its suitability for specific PC recipes (Guinee, 2009). It is essential to adjust the young and aged cheese ratio to ensure that PC has a certain amount of intact casein. When young NC (with high intact casein) is used to make a PC, the hardness of the PC is increased, and the meltability is decreased (Templeton and Sommer, 1932). To make block PC, which has high scalability and elasticity, young cheese (75-90% intact casein) is utilized; PCS is made by utilizing mature or aged cheese (60-75% intact casein) (Fox et al., 1996).

2.1.4.2 Emulsifying salts (ES)

ES are ionic compounds made of monovalent cations and polyvalent anions. The two principal roles of ES in PC are calcium sequestering (assisting in disrupting the calcium–phosphate-linked protein network seen in NC during PC formation) by ionic exchange reaction and pH adjustment. Both capabilities contribute to the hydration of the caseins found in a NC, allowing them to interact readily with the water and fat phases resulting in a homogenous process of cheese emulsion (Chen and Liu, 2012; Schatz et al., 2014; Sołowiej et al., 2020). The FDA has approved the use of 13 ES in producing PC (either individually or in a mixture) (21CFR133.169 - 133.180). This includes salts of mono-, di-, trisodium phosphates, sodium hexametaphosphate, sodium acid

pyrophosphate, tetrasodium pyrophosphate, sodium aluminum phosphate, sodium citrate, potassium citrate, dipotassium phosphate, calcium citrate, sodium potassium tartrate, and sodium tartrate as shown in Table 1. Trisodium citrate and DSP are the most common ES in the U.S. to produce PC.

On the use of ES in PC and their effects on functionality and cheese chemistry, several studies have been conducted (Kapoor et al. 2008; Gupta et al., 1984; Caric and Kalab, 1993; Kosikowski and Mistry, 1997; Shirashoji et al., 2005; Tamime, 2011). However, the conclusions of these investigations vary owing to the varied experimental designs employed. The manufacturing conditions, age of NC, and quantity of ES vary. Due to the disparities in experimental design, it is challenging to compare the diverse study undertaken.

2.1.4.3 The role of pH on the functionalities of PC

The pH of PC significantly influences its structure and functional qualities. The various textures of PC are made with different pH levels (4.8 to 6.0). To the extent that only monosodium citrate and monosodium phosphate are used as the only salt emulsifying agent, short, dry, crumbly, and prone to oiling-off cheeses with low pH (4.8–5.2) are produced, whereas PC with high pH (>6.0) tend to be incredibly soft and flow excessively on heating (Templeton and Sommer, 1932). Melted PC loses hardness and storage modulus (G′) when pH rises from 5.7 to 6.2 but gains flowability and fluidity (loss tangent) (Lee and Klostermeyer, 2001). CN hydration has increased, paracasein aggregation has decreased, and the gel network is more acceptable and homogeneous (Guinee, 2009). The pH of NC ranges between 5 to 5.5; however, the pH of PC is ranged

between 5.6 to 5.9. The pH of PC plays a critical role in the PC's functionalities, such as melting, hardness, textural, and binding capacity of casein. A study has been made that examines different ES with varieties of pH ranges of PC (5.3, 5.6, and 5.9) (Marchesseau et al., 1997). The study shows that with higher pH: i) The electrostatic repulsion of the CN matrix will increase. Between the pH of 5.2 to 5.7; ii) the charge of CN was more negatively high with increasing the pH; iii) The PC network will be more open and give a better binding capacity of CN and the emulsifying ability of ES during PC processing; iv) The sequestration of calcium from the CN network by ES will be increased; v) The hydrophobic interaction between CN will be decreased.

2.1.4.4 Lactose level

Lactose is also significant for its economic worth and its contributions to the sensory and functional aspects of dairy products. Lactose is a cheap dairy ingredient in PC formulation that could increase the profit of PC, but it could increase some chemical reactions in PC. The lactose concentration of PC is another essential formulation parameter that must be regulated in a PC formula since a greater concentration of lactose in PC may produce lactose crystal formation and a non-enzymatic browning reaction. The main lactose contributors in a PC formula are nonfat dried milk (NFDM) and dry whey. Several studies have focused on the issue of lactose crystallization in PC caused by the use of NFDM or whey powder (Templeton and Sommer, 1932, 1934; Berger Klostermeyer, Hargreaves, Gillian., 1998). Lactose crystallization in PC is determined by the highest concentration of lactose soluble in the PC water phase (Templeton and Sommer, 1932; Thomas, 1973). At 20°C, the maximum soluble lactose concentration in water is 17% (Templeton and Sommer, 1932; Zadow, 1992). As a result,

it is critical to keep the quantity of lactose in the water phase of PC to less than 17% to prevent lactose crystallization. As a result, while developing PC, producers should guarantee that the final lactose level does not exceed 7.48% for PC (44 percent moisture product) and 10.20% for PCS (60% moisture product) (Kapoor and Metzger, 2008). Another flaw resulting from including lactose-rich components in PC is non-enzymatic browning, which results in undesirable color and taste development (Thomas, 1969). According to Thomas (1969), post-manufacture storage temperature and ripening as well as the pH of PC all substantially impacted the browning of PC. He recommended that PC not be kept at temperatures over 35°C for longer than 6 weeks.

2.1.4.5 Whey protein content

Bovine milk has 80% CN and 20% whey proteins (Séverin and Wenshui, 2005). About 80% of milk's whey proteins are β-Lactoglobulin (β-LG) and α-lactalbumin (α-LA) that are the two main whey proteins. β-LG has a "reactive" free sulfhydryl group in their main structure (Wong et al., 1996). Whey proteins denature at 60 to 70°C. This high-temperature denaturation of β-LG exposes the free sulfhydryl group, which may form disulfide connections with β-LG and κ-CN (Euber and Brunner, 1982). NFDM and whey protein concentrate (WPC) in PC composition may boost whey protein levels. Since whey proteins may crosslink with caseins at high temperatures, a high amount of whey proteins in the PC recipe might affect its sensory characteristics, hardness, and meltability. The influence of whey protein incorporation in PC on its functional and sensory properties has been studied extensively (Gupta and Reuter, 1992, 1993; Abd El-Salam et al., 1996; Fayed and Sonia, 1999). Whey was ultrafiltered to generate a liquid concentrate (26% TS) with 20% protein and 5.8% lactose to replace 20% of the solids in

a PC formula. They found that adding up to 8% whey protein to PC with an average moisture level of 47% did not affect PC's overall acceptability score. Thapa and Gupta (1992) found that PCF, including WPC (at the same final whey protein content), was stiffer than PCF without WPC. Abd-El-Salam et al. (1996) evaluated the influence of liquid WPC (28% TS, 15% whey protein) on the compositional and rheological characteristics of PCS (57% moisture, 3% ES). Adding WPC to PCS increased moisture by 0.8%, lactose by 2.5%, and pH by 0.3 compared to PCS without WPC. Meltability, taste, and sensory qualities of PCS improved as WPC in PCS increased (perhaps owing to increasing moisture content). Mleko and Foegeding (2000) demonstrated that up to 2% of rennet casein PC might be substituted with whey protein. However, PC became firmer and less meltable. Heat-induced disulfide connections between β-LG (in whey protein) and κ-CN (in rennet casein) affect PC's firmness and melting qualities. In the rennet casein-based model PC, casein proteins were replaced by polymerized whey protein (Mleko and Foegeding, 2000). They introduced polymerized cross-linked whey protein to a model PC by heating whey protein dispersions to elicit disulfide crosslinks. As the quantity of polymerized whey protein grew, PCP became firmer and less meltable. In a 17% rennet casein-based PCP system, replacing 4% rennet casein with 2% whey protein polymers PC with the same texture and meltability (Mleko and Foegeding, 2001).

2.1.4.6 Total calcium content

The total calcium level of PC determines its ultimate functional qualities and plays a role in its manufacturing. Higher total calcium content in PC formula complicates PC production because more calcium must be sequestered from NC caseins by ES added during PC production (Sood et al., 1979; Caric et al., 1985; Cavalier-Salou and

CHEFTEL, 1991; Zehren and Nusbaum, 2000). The total calcium content of cheeses with varying fat levels differed substantially. It is critical to examine calcium levels when examining cheese proteolysis and functioning since calcium is vital in the cheese body and cheese meltability (Dave et al., 2003). Cavalier-Salou and Cheftel (1991) discovered that when the calcium concentration of cheese analogs increased, their hardness improved, and their meltability reduced in research on cheese analogs utilizing sodium caseinate. NC is the main element that leads to changes in the overall calcium level of a PC composition. It has been discovered that using NC with a high total calcium level to manufacture PC results in a firmer, less meltable PC (Zehren and Nusbaum, 2000).

2.2 Manufacture of PC

Similar manufacturing processes are involved in producing various PC, including selecting NC and materials, formulation, shredding of NC, blending, processing, packing, cooling, and storage. They are described in the details below:

2.2.1 *Selection of natural cheese and ingredients*

The maturity and type of NC are selected based on the desired characteristics of the final product. In terms of maturity, young cheese can help lower the cost of materials and has a greater capacity to bind water; however, it may alter the final product's flavor. It cannot be argued that choosing the right cheese is a critical factor that plays a vital role in PC's physical and chemical properties. NCs vary in processing, compositions, pH, parentage of intact casein, and calcium, as shown in Table 2. These distinctions directly affect PC functionality. Processed Cheddar cheese is famous in the United Kingdom and

Australia; Cheddar, Gruyere, and Mozzarella are famous in the United States and Canada; while Emmental is widespread in Western Europe (Carić and Kaláb, 1999).

2.2.2 Formulation

Ingredients are estimated based on recognized NC components' fat and dry matter. The material balance of fat and dry matter must be determined so that the completed product has the proper composition. This includes all blend materials, additional water, and condensate from direct steam used during processing. Additional adjustments to the fat and dry matter can be made prior to completing the processing, as shown in Table 3. Some ingredients have limitations to avoid any change during the storage period that could reduce the quality. The percentage of lactose should be less than 6% in the final product to avoid the Maillard or crystallization reactions. Table 4 shows that the amount of NC in the formulation has varied based on the type of PC.

2.2.3 Shredded natural cheese

This procedure improves the interaction between ES and the mixed ingredients during processing. Shredding, grating, or mincing the additional cheese increases the surface area of the cheese and facilitates heat transmission during further processing (Guinee, 2009).

2.2.4 Blending and processing

All dairy ingredients (NC, butter, and MPC) were combined with nondairy ingredients using the blending system (color, flavor, salt, and water). Additionally, ES can be added to the ribbon blender or during processing. The formulation components'

mixing guarantees that all elements are homogeneous and that the finished result has a consistent quality. The term "processing" refers to heating (75 to 85°C) the mix using direct or indirect steam in a partial vacuum with continuous agitation for 1 to 5 mins. There are two fundamental types of cooking apparatus: 1) circular (double-jacketed kettle, up to 200 liters), and 2) tube-shaped (about 4 meters in length, equipped with one or two mixing worms) (Carić and Kaláb, 1999; Guinee et al., 2004). The blending and processing are critical steps of processing the PC in terms of eliminating any harmful or spoilage microorganisms, hence extending the shelf life of the finished PC. This encourages the interaction of NC with other ingredients, such as ES, water, and fat, and promotes the physicochemical and microstructural changes necessary to convert the mix into a final product with the appropriate properties and physicochemical stability (Guinee, 2009).

2.2.5 Packaging

It is a critical stage in the processing of PC. After the cooking phase, a hot PC can be supplied to the packing machine at a temperature of 60-80°C and cooled 25-35°C for 30-60 mins. PC can be filled in glass jars, tubes, cans, and foil. For PC spread, the cheese can be sudden cooling.

2.2.6 Cooling and storing

PC should be stored at temperatures below 10°C to induce fat crystallization and modulate the kind and degree of protein interactions between the fat globules coated with paracaseinate and the scattered paracaseinate molecules. Also, it encourages the required

degree of setting and creation of the final product's textural features to the degree controlled by the cooling rate.

2.3 Functional properties of processed cheese

The required functional features of PC may be divided into two groups based on their end-use application: unmelted texture properties and melted texture properties. Specific PC applications demand excellent interplay between melted and unmelted textural features and specific functional properties. As a result, the functional qualities needed for each PC are distinct (Kapoor and Metzger, 2008). Researchers have developed many empirical and instrumental methodologies to assess and quantify the functional qualities of PC.

In addition, PC functionality may be classified into three broad categories: features linked to the rheology of raw cheese (fracture characteristics), cooking properties (flowability), and flavor/aroma properties (Guinee, 2011). The ingredients of PC include fat, moisture, intact casein, ES, and pH impact the functional properties of PC as shown in Table 5.

2.3.1 Firmness

Firmness measures a product's degree of hardness, measured by the gram. Templeton and Sommer (1930) conducted one of the early investigations on the influence of several factors on the firmness of PC. The hardness of PC was determined by compressing a standard PC sample to a defined height and measuring the force

exerted in grams. The hardness of PC decreased with decreasing level of intact casein in NC, pH, level, and type of ES used in the formulations.

2.3.2 Meltability

Meltability is the ease with which cheese flows or spreads when heated by the composition of ES and the maturity degree of NC used with PC manufacturing (Muthukumarappan et al., 1999; Acharya and Mistry, 2007; Brickley et al., 2007). Meltability is determined by the melt's solid cheese's heat transport and thermal phase transition properties and the rheological or flow properties (Park et al., 1984). Schreiber (Kosikowski and Mistry, 1977), Amott (Amott et al., 1957), and Dynamic stress rheometer (DSR) (Hammam et al., 2021b) described the most widely reported meltability assessment methods for PC. Schreiber and Amott's approaches rely on cooking a standardized cylindrical cheese specimen under specific conditions (oven temperature and duration), then measuring the specimen's height drop (Amott) or diameter expansion (Schreiber) (Park et al., 1984). Some disadvantages of the Schreiber test are that the cheese spreads are not spherical, and the spread's outer edges are burnt (Park et al., 1984). These flaws lead to mistakes in this test, which is already empirical, resulting in unpredictable results. On the other hand, the Schreiber test is still widely used because of its simplicity (Muthukumarappan et al., 1999).

2.3.3 Viscosity

When PC is melted, viscosity refers to the propensity of polymer to spread and flow. The amount of NC and age, manufacturing mixing speed, and ES concentration substantially influence PC viscosity. As young NC contains more intact casein than

mature NC, PC should have more extensive protein-protein and protein–fat interactions, resulting in a greater viscosity after manufacturing. Viscosity is essential in PC since it determines the finished product's flow when melted (Guinee, 2002). The flow of melted cheese might decrease as its viscosity decreases.

2.4 Methods of measuring processed cheese properties

2.4.1 Textural Profile Analysis (TPA)

TPA is a frequently used technique for determining unmelted textural qualities, including hardness, adhesiveness, springiness, cohesion, and gumminess. TPA hardness measures the unmelted cheese texture, which encapsulates the cheese's firmness (Breene, 1975; R Kapoor et al., 2008). Drake et al. (1999) discovered a strong relationship between G', G" and TPA firmness. According to Gupta and Reuter (1993), the decreased PC penetration values with increasing WPC concentration are mainly attributable to higher whey protein, which becomes denatured during further heat processing.

2.4.2 Dynamic rheology

The viscoelastic properties of cheese may be determined using dynamic stress rheometer (DSR), which helps researchers better understand the cheese's unique properties (Lucey et al., 2005). This test measures the G' (storage modulus) and G" (loss modulus) that quantifies the energy wasted in each deformation cycle; and the tan δ that is G″/ G′. Tests performed at the same frequency and strain over an extended period are frequency sweeps (strain or stress constant) at a fixed temperature or temperature (Gunasekaran and Ak, 2002). Sutheerawattananonda and Bastian (1998) created a DSR-

based approach for PC that involves heating the sample and using DSR to determine the G', G", and melting temperature. As tan δ increases, the material becomes more viscous and less elastic in response to an external force. The crossover modulus is the value at tan δ = 1 when the material exhibits similar solid and liquid-like properties. Tan δ less than one indicates that the material is more elastic; tan δ more than one indicates that the substance is more viscous.

2.4.3 Rapid Visco analyzer (RVA)

The Rapid Visco Analyzer (RVA) has been used effectively in PC manufacturing on a small scale (Metzger et al., 2002; Kapoor and Metzger, 2005; Hori et al., 2016). Newport Scientific (Warriewood, Australia) developed the RVA computer-integrated apparatus for testing the viscosity of foods. The RVA can analyze apparent viscosity under operator-specified shear and temperature conditions. Moreover, the apparent viscosity after RVA production is associated with the functional features of the PC. RVA may be used as a production and analytical technique on a small scale to anticipate PC's functional qualities and assess how different formulations and processing factors impact these functional features.

2.5 Phosphorylation of lactose

2.5.1 Lactose

Milk is the only natural source of lactose that ranges between 0-10% depending on the age, health, stage of lactation, nutrition, and type of mammals (P. F. Fox et al., 1998). The primary carbohydrate in the milk of most mammals is lactose, commonly

known as "milk sugar." Whey permeate, a byproduct of cheese and casein production, is used to make lactose commercially. It is widely used in food and pharmaceutical industries because it is a cheap ingredient, source of low sweetness, and filler (Lifran et al., 2000).

2.5.1.1 Chemical and physical properties of lactose

Lactose, a disaccharide carbohydrate ($C_{12}H_{22}O_{11}$), is the mean carbohydrate in milk. Lactose is composed of a D-glucose and a D-galactose linked by β-1, 4 glycosidic bonds (Figure 1). There are six different lactose polymorphs and two anomers, α or β-lactose (Holsinger, 1988). The crystalline structure of anhydrous β-lactose, anhydrous α-lactose (unstable), anhydrous α-lactose (stable), complex β/α lactose, and the amorphous structure composed of a 5:4 ratio of α and β-lactose are other polymorphs of lactose (Drapier-Beche et al., 1997; Lara-Mota et al., 2021). The ring-opened can be an α or β form depending on the hydroxyl group at carbon 1 in the glucose part, affected by temperature, pH, moisture percentage, and solvent type (Walstra and Jenness, 1984).

Lactose is the lowest soluble sugar compared to fructose and sucrose (Andreeta, 2012). These two anomeric forms make lactose solubility less soluble than other sugars (Anantakrishnan and Herrington, 1948). The temperature, specific salt, alcohols, whey, and other sugars play a role in the solubility of lactose (Nickerson and Lim, 1974; Huang et al., 1988; Bhargava and Jelen, 1996). At 20°C, the solubility of α and β lactose is approximately 70 and 500 g/L, respectively (Zhang et al., 2015b). Therefore, α-lactose has more solubility at > 93.5°C. α-lactose crystalizes as a monohydrate at < 93.5°C;

however, β-lactose crystalizes at > 93.5°C as anhydrous crystals that make the α-lactose more familiar form to crystal (Fox et al., 2015), as shown in Figure 2.

The structure of these particles has different shapes depending on saturation level, temperature, presence of impurities, and physical properties, including density melting point and refractive index of lactose (Mcdonald and Turcotte, 1948; Michaels and Vankreve, 1966; Jelen and Coulter, 1973). The crystallization yield of α-lactose is 5% greater than β-lactose (Fox, 2008). Lactose is a reducing sugar to an aldehydic role in the ring-opened form of glucose. Lactose has low sweetness compared to sucrose (30% of sucrose sweetness) (Vaclavik et al., 2021).

2.5.2 Mechanisms of lactose crystallization

The crystallization process is commonly used in food and dairy industries to separate and purify components such as lactose and fat. The crystallization is divided into two stages: nucleation and growth of the nucleus (Wong and Hartel, 2014). These two processes create a complicated process determined by the nucleation and growth rates (Shi et al., 1990). Controlling final product properties such as median size and particle size distribution is challenging because of the many interactions between lactose saturation, solubility, mutarotation, nucleation, and growth. Additionally, contaminants in the crystallizing solution significantly affect the final result (Lifran, 2007).

2.5.2.1 Nucleation

The nucleation process forms a crystal (nuclei), referred to as the first step. It takes the form of a solid crystalline phase in a supersaturated solution (Poehlein and

Wenzel, 1972). The proliferation of nuclei is environment-dependent and may be accelerated by increasing the available saturation. Clustering, molecular aggregation, ions in solution, vapor, and supersaturated melt are all variables that may aid in nucleation.

The nucleation can be divided into two steps: primary, which can be occurred either in the presence (heterogeneous) or absence (homogeneous) of suspended particles, and secondary, which can only occur with the presence of seed crystals (Bhargava and Jelen, 1996). The secondary nucleation has low activation energy that can be occurred at a low level of saturation compared to primary nucleation (Mcleod, 2007). Many types of secondary nucleation such as contact nucleation, initial breeding, fracture, and dendritic separation (Shi et al., 1989). Induction time is the needed time to switch from initiation saturation to the formation of nuclei that can be affected by solution concentration. The induction time is known as the rate id nucleation.

2.5.2.2 Crystal growth

The crystal growth can be separated into three steps (Hartel and Shastry, 1991). First, mass transport changes from the bulk to the crystal surface. The second is a surface reaction, which diffuses lactose molecules on the surface. The third is the transport of latent heat, which moves latent heat away from the growing crystals. Many factors affect growth, such as process parameters and solution characteristics, including saturation, impurities, temperature, viscosity, and pH (Bhargava and Jelen, 1996).

On the other hand, Hartel (1966) divided the growth into eight steps: 1) Spreading the lactose molecules to the solid interface; 2) Changing the molecules to an

appropriate anomeric shape for growth; 3) Removing all or some of the water from the lactose molecule; 4) Diffusing and moving away from water molecules; 5) Tending the lactose molecules to the adsorbed layer between the solution and crystalline phase; 6) Diffusing of lactose molecules on the surface; 7) Incorporating the growth unit into the crystal lattice, and 8) Removing any heat release during the above phase change.

2.5.3 Manufacturer of lactose

Lactose is a versatile ingredient having numerous applications in the food and pharmaceutical industries. Ice cream, yogurt, cheese, infant formula, reconstituted dairy products, bakery, and confectionery goods are typical examples of lactose in the food and dairy industries. Overall, manufacturing lactose crystallization involves numerous unit operations such as filtration, thermal treatment, evaporation, and drying. The number of crystallizations is used to classify the lactose as edible lactose or pharmaceutical grade α-lactose monohydrate. Figures 3 and 4 illustrate flow diagrams for manufacturing edible lactose and pharmaceutical grade α-lactose monohydrate, respectively. Pharmaceutical grade α-lactose monohydrate is involved two times purifications. It has the purest lactose compared with edible lactose. Lactose crystallization in the industrial setting is often accomplished using a slower batch cooling method, which results in edible grade lactose that contains a large amount of non-lactose components. A second crystallization procedure is required to purify lactose to pharmaceutical grade (Hartel and Shastry, 1991).

2.5.3.1 Clarification

Whey protein can contain fat, casein, and some impurities such as foreign particles and somatic cells that affect the quality of lactose products. Thus, clarifying the whey as soon as been received is crucial to avoid any physical or chemical change in the final products. The whey clarification can be done by passing the whey through a rapidly rotating clarifier under pressure at 10°C using centrifugal separators (Singh and Singh, 2015). After that, the whey is cooled and stored at 4°C.

2.5.3.2 Ultrafiltration

Ultrafiltration processing has been used in the food and dairy industries to purify whey protein (Baldasso et al., 2011). It can separate the lactose and minerals (permeate) from whey protein bypassing the solution through the membrane.

2.5.3.3 Evaporation

The evaporation is typically done under low pressure to decrease the boiling point temperature. The lactose is evaporated between 50 to 70°C to obtain 60 to 65% TS (Singh, 1992). The concentrated lactose should not stay warm for a long time to prevent the microorganisms from growing (Palmer, 2010).

2.5.3.4 Drying

Many dryers have been used in the dairy, food, chemical, and pharmaceutical industries, such as spray, roller, freeze, microwave, and steam dryers (Smithers and

Augustin, 2013). The fluid bed dryer is involved drying the lactose concentrated by flowing the hot air to the product on the perforated steel bed.

2.5.4 Maillard reaction (nonenzymatic browning)

Maillard reaction is a chemical reaction that Louis Camille Maillard discovered in 1912. It is a generic term that refers to nonenzymatic browning caused by the reaction of reducing sugars and proteins. PC's moisture level and water activity (a_w) significantly affected the Maillard process (Hyslop, 2003). The Maillard reaction occurs when a carbonyl functional group in a reducing sugar reacts with the α-amino group in amino acids (lysine).

The nonenzymatic browning reactions are influenced by the amount of water in PC and their a_w (Hyslop, 2003). The maximum response rate is obtained when the a_w is between 0.60 and 0.85. The Maillard reaction is strongly dependent on water content (Nascimento et al., 2021). When the moisture level is between 30% and 75%, the reaction happens readily; the reaction rate rises as the moisture content increases (Xiang et al., 2021). The reaction occurs when the carbonyl compounds combine with the amino acids to generate an unstable Schiff base, a reversible reaction in the Maillard reaction. Then, via double bond migration and rearrangement activities of the Schiff base, the reduction efficiency may be increased during the development of relatively stable Amadori or Heyns rearrangements (Cristina De Oliveira et al. 2016). The intermediate step of the Maillard reaction entails Amadori product degradation, notably sugar dehydration and deamination, Strecker degradation, and sugar fragmentation (Xiang et al., 2021). Since

water is a byproduct of the Maillard process, the a_w may grow as the Maillard process occurs.

2.5.5 Phosphorus

In the human body, phosphorus is found in the bones (85%), the soft tissues (14%) and the extracellular fluids, intracellular structures (1%), and cell membranes (0.01%) of the total phosphorus (Nielsen, 2010). Food phosphorus is a compound composed of inorganic phosphate (Pi) and various organic phosphates. Because intestinal phosphatases hydrolyze the organic forms in meals (IOM, 2018), most phosphorus is absorbed as Pi, and the predominant form of Pi in all biological fluids and tissues is the divalent anion hydrogen phosphate ion (HPO_4^{2-}). Phospholipids, nucleotides, and nucleic acids are composed chiefly of organic phosphates. In addition, the calcium-to-phosphate ratio in the hydroxyapatite-like bone crystals is around 2:1. In contrast, the Pi compartment of the whole body contains a minute proportion of total body phosphorus and is primarily contained in the blood and extracellular fluid. Nevertheless, the Pi compartment is an essential tool because it takes phosphate ingested from the food and phosphorus resorbed from bone and is the source of most bone fluid phosphorus and the majority of urine phosphorus (McSweeney and Fox, 2009). The bioavailability of phosphorus in most dietary sources ranges from 55 to 70% (IOM, 2018). Phosphate salts are added to PC for non-nutritive activities such as moisture retention, smoothness, and binding, increasing the phosphorus content of the U.S. food supply. In order to induce dietary phosphorus shortage, which presents as hypophosphatemia, near absolute fasting is necessary. In bovine milk, the soluble portion of Pi is around 54%, whereas the insoluble portion is approximately 46%. Pi is connected with casein micelles as calcium

phosphate, most likely due to binding primarily to casein phosphoserine residues (organic phosphate) and glutamate and aspartate residues (Gaucheron, 2005).

2.5.6 Lactose phosphate

LP is a natural compound that switches the hydrogen on lactose by monophosphate. Thus, lactose and lactose phosphate are structurally identical. Typically, LP is separated as a barium salt or a free acid (MacDonald, 1972). The bulk of lactose phosphate molecules has a phosphate group attached to the lactose galactose moiety. Around 90% of LP in pharmaceutical-grade lactose is connected to galactose; 10% of LP is attached to glucose (Breg et al., 1988). The monophosphate might be placed on the glucose moiety's carbon 6 or the galactose moiety's carbon 3, 4, or 6, as shown in Figure 5.

LP is a compound naturally present in milk and milk products with various percentages (Table 6). LP was discovered for the first time in bovine milk (Barba and Caputto, 1965) and was recently discovered in caprine milk (Albrecht et al., 2014). A fundamental understanding of LP chemistry is required to develop a technique for the isolation and purification of LP from lactose and the development of an analytical method to determine this compound in lactose and other milk.

Lactose monophosphate is a dibasic acid with two greater pH values than phosphoric acid: pKa1= 1 and pKa2= 6 (Visser and RA, 1980). LP is neutral at very acidic pH values of less than 1. At pH 3 to 4, 99% of LP is monovalent, as present in Table 7. It is well established that under the influence of acids, phosphate esters of polyhydroxy compounds undergo intramolecular migration of a phosphate group from

one OH group to another (Finelli et al., 2002). This migration most likely occurs through a cyclic intermediate, resulting in equilibrium mixes of isomeric phosphate esters (MacDonald, 1972).

Lactose is used in the research to refer to the most common commercial form of lactose and α-lactose monohydrate. During the process of crystallization, the lactose crystals exhibit a surprising feature. Biopolymers such as oligonucleotides and dextran may be incorporated into them, and proteins, salts, LP, and other compounds (Lifran, 2007). LP is different from these chemicals in that it has a distinct function (Alsaleem and Metzger, 2022). Despite being present in insufficient quantities, it has a significant detrimental impact on the development of lactose crystals. There have been no published studies on the effects of LP on lactose crystallization in the circumstances relevant to the industrial scenario, despite the significance of these findings. One reason for this might be the difficulty in getting pure lactose, which could be used as a valid reference, and an economical supply of LP.

The proportion of LP that integrates into the final crystals varies depending on the quantity of LP present at the start of the crystallization process. The percentage of LP that integrates into the final crystals was between 60 percent and 80 percent of the original amount of LP (Lifran et al., 2007). Using LP in PC as ES could help to increase the amount of lactose in PC formulation (Alsaleem et al., 2021). However, this could affect the functionality of PC when lactose% water is higher than 17%.

2.6 Milk permeate

Milk permeate (MP) consists of water and milk solutes of low molecular weight, including minerals, vitamins, whey proteins, and lactose (Fitzpatrick and O'Keeffe, 2001). The bulk of the total solids in MP is lactose. Massive quantities of milk permeate generated by the dairy industry have shifted from being a significant waste concern to being employed in various ways to provide value to the sector. In the dairy industry, considerable quantities of MP are produced by the ultrafiltration (UF) process used to concentrate milk's fat and protein content (Cuartas-Uribe et al., 2009).

MP is employed in animal feed, land fertilizer, and lactose powder manufacturing (Parashar et al., 2016). Since the Codex Alimentarius Commission permitted the standardization of milk's protein content in 1999, permeate has been used extensively as a mixing agent in the protein standardization of milk (Tsermoula et al., 2021). Due to the significance of permeate as an ingredient, it is crucial to recognize and regulate the compositional fluctuation to maintain the consistent quality of standardized milk, as shown in Table 8.

Moreover, MP is used in baked foods, meats, soups, confectionery, dry mixes, and drinks for a variety of functional and nutritional purposes, including i) a Cost-effective approach for reducing salt levels; ii) browning of baked products caused by the Maillard reaction of lactose and other reducing sugars, which enhances flavor and imparts a pleasing caramelized taste; iii) the improved emulsification of the fat in the formulation and the improvement in water-holding capacity contributed to the

preservation of softness Soups' flavor and texture; and iv) removing sweetness and imparting crystalline properties to confections (Henning et al., 2006; Jana, 2017).

2.6.1 Manufacture of milk permeate powder

Manufacturing MPP involves numerous unit operations such as standardization, thermal treatment, membrane filtration, evaporation or concentration, and drying. Figure 6 illustrates a flow diagram for MPP manufacture.

2.6.1.1 Standardization

Standardization of milk is the first stage in the dairy industry when milk is received. Numerous dairy businesses get milk from various sources, including varying proportions of fat and non-fat components. This may result in producing goods of varying quality and decreasing profit. Standardization is thus a process for adjusting total solids (TS) to meet product specifications. The fundamental goal of standardization in the production of MPP is to eliminate as much milk fat as possible so that it may be utilized for various applications.

The primary goal of standardization in the production of whole milk powder (WMP) is to standardize fat to TS or non-fat (Ipsen and Hansen, 1988). For the production of WMP, just protein is extracted, whereas, for the production of skim milk powder (SMP), fat, lactose, and salts are extracted (Singh and Singh, 2015). For SMP production, the MF should not exceed 0.01% fat. The milk fat content of SMP and WMP should be 1.5% and 26-40%, respectively, in the final product.

2.6.1.2 Membranes filtration

Microfiltration, ultrafiltration, nanofiltration, and reverse osmosis membranes are among the types of membrane filtration employed in milk. Particular pore size is assigned to each membrane type following the size of the components that need to be separated (Le and Nunes, 2016). A membrane's nature or properties, operating pressure, and pore size all have an essential role in using it for a specific purpose. Milk is processed with microfiltration to remove large particles such as bacteria, fat, and micellar casein. Protein concentration, lactose removal, and soluble minerals from dairy products are all common ultrafiltration uses. The removal of monovalent salts and water is the only purpose of nanofiltration. Reverse Osmosis reduces the volume, recovers the total solids, and removes the water from the product.

This technology makes it possible to produce by-products that can be used to improve the current or developed new ones. WPC and WPI can be produced using the membranes filtration from the whey that remains from cheese manufacturing. Also, it is possible to recycle the water and use it again for the cleaning side.

Ultrafiltration processing has been used in the food and dairy industries to purify whey protein (Baldasso et al., 2011). It can separate the lactose and minerals (permeate) from whey protein bypassing the solution through the membrane.

2.6.1.3 Concentration of milk permeate

Typically, evaporation is conducted at low pressure to lower the boiling point temperature. Between 50 and 70°C, the milk is evaporated to provide 40 to 50 percent TS

for spray dryers and 30 to 35 percent TS for roller dryers (Singh, 1992). This temperature range is optimal for minimizing the denaturation of whey protein (Sandu et al., 1991). The condensed milk should not be heated for an extended period to prevent the development of bacteria (Walstra, P., Geurts, T.J., Noomen, A., Jellema, A., 1999). The primary objective is to remove water to preserve the quality of MP in the face of increased viscosity, which may make removing water from concentrated milk challenging. It also helps to reduce the energy needed for spray drying (Schuck, 2011).

In contrast, ultrafiltration and reverse osmosis filtration are techniques that may be used to concentrate milk to produce high-protein milk powders (Elgazzar and Marth, 1991). Therefore, just pasteurization is required for MPC production (Singh and Singh, 2015). The ultrafiltration membrane enables only water, lactose, non-protein molecules, and a few soluble salts to pass through.

2.6.1.4 Drying

Spray, roller, freeze, microwave, and steam dryers have been used in the dairy, food, chemical, and pharmaceutical sectors (Smithers and Augustin, 2013). Due to its higher solubility, quality, and color, spray drying is often utilized for MP. The spray dryer is responsible for atomizing concentrated milk into hot air. By putting the concentrated milk into a spray of tiny droplets and exposing it to a flow of hot air (inlet 80 – 90°C, output 180–220°C), the milk is dehydrated (Pisecky, 1997). It might be a pressure nozzle or a centrifugal disc. The tiny droplet size expedites evaporation at low temperatures and reduces powder damage (Refstrup, 2003). The powder gathers at the cyclone's base. It is necessary to concentrate the milk to reduce the drying energy since

this is a costly process. The spray dryer may have either one or two phases. A single-stage system takes less time and has a greater exit temperature than a two-stage system (Smithers and Augustin, 2013).

In contrast, the roller drier dries concentrated milk by exposing it to the heated surface of the rolling rollers. A thin coating of condensed milk (1.1 mm) is dried at 100°C by a rotating metal cylinder or a drum. Due to its expense and unwanted effects on milk powder, such as caramelization of the lactose and denaturation of the protein, this process is not desired and is seldom used. Also, the freeze dryer is used to freeze a thin layer of milk at -20°C under a vacuum (Wang et al., 2004). This procedure is costly, unsuitable for significant amounts, and causes fat globules to agglomerate.

2.7 Activated carbon

High-carbon-content activated carbon created from environmental waste is the essential substance for removing environmental contamination (gases and liquid impurities). Additionally, the usage of activated carbon has been expanded to include decolorization, gas separation and polluted air treatment, heavy metal recovery, and food processing with no hazard (Garrido et al., 1992; Bernardo et al., 1997; Abdulkarim et al., 2002). Producing activated carbon from any solid carbonaceous precursor, whether natural or synthetic, is possible. The selection of precursors is heavily influenced by their availability, cost, and purity. Due to environmental concerns, agricultural wastes are regarded as a highly essential precursor since they are inexpensive, renewable, safe, readily accessible in huge numbers, and easy to identify; they have a high concentration of carbon and a low amount of ash. For the preparation of activated carbon, various

polymeric wastes derived from petroleum, agricultural byproducts (lignocellulosic), and coals are often utilized as starting materials (Olivares-Marín et al., 2012).

The qualities and attributes of activated carbon are dependent on the physical and chemical properties of the starting materials and the activation techniques (Tiryaki et al., 2014). Powder activated carbon (PAC), granular activated carbon (GAC), and fibrous activated carbon (FAC) are the three forms of activated carbon (ACFs) (Zaid et al., 2013). Physical properties of activated carbon, such as surface area and bulk density, and chemical properties, such as pH, ash content, and conductivity, may influence the substance's usage and acceptability for specific applications (Castro et al., 2000). For the adsorption of organic chemicals such as methylene blue (MB) and phenol (Ph), large surface area, high bulk density, neutral pH, low ash, and low conductivity are often preferred. Activated carbon two substantial adsorption porosity has led to its widespread usage as an adsorbent and catalysis and separation processes.

Physical and chemical activation are the two fundamental ways of creating activated carbon. Physical activation, also known as thermal activation, consists of two steps: activating starting material and activating char (500- 900°C) with carbon dioxide or steam. Pyrolysis is the heat decomposition of an organic substance in a vacuum or inert environment. Complex, contemporaneous, and sequential reactions occur throughout this process, giving birth to three fractions: fixed carbon mass (charcoal), pyroligneous liquid, and gaseous products (H_2, CO_2, CO, CH_4). The starting components are impregnated with an activating substance during chemical activation ($ZnCl_2$, NaCl, Na_2CO_3, K_2CO_3, KOH, NaOH, Al_2O_3, H_3PO_4, H_2SO_4, NH_4C) (Ahmed and Theydan,

2012). Porosity and surface area of activated carbon significantly impact its adsorption capacity (Tongpoothorn et al., 2011).

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Tables

Table 1. Chemical characteristics of emulsifying salts used in processed cheese.

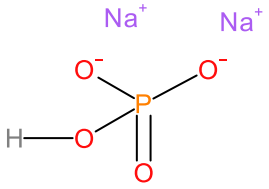
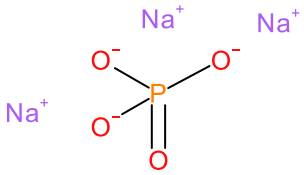
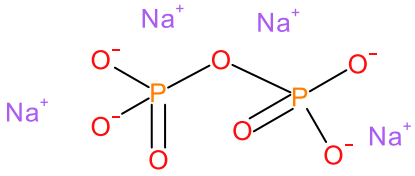
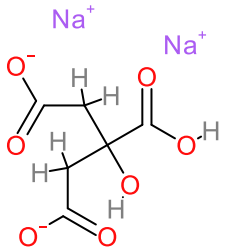
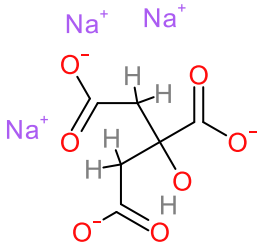
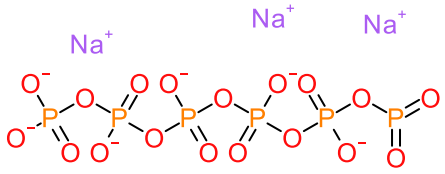
Name	Empirical formula	Molecular weight	Chemical structure
Disodium phosphate	Na_2HPO_4	141.96	
Trisodium phosphate	Na_3PO_4	163.94	
Tetrasodium pyrophosphate	$Na_4P_2O_7$	265.9	
Disodium citrate	$Na_2C_6H_5O_7$	236.09	
Trisodium citrate	$Na_2HC_6H_5O_7$	258.07	
Sodium hexametaphosphate	$Na_6O_{18}P_6$	611.7	

Table 2. Chemical composition of various natural cheese.

Type	Fat	Protein	Calcium	Moisture	pH
Edam	27.80	25.00	0.73	41.40	5.70
Cheddar	31.50	25.40	0.72	37.00	5.10 – 5.60
Swiss	28.80	28.90	1.10	36.00	5.5-5.7
Mozzarella	21.70	19.40	0.52	54.10	5.20
Gouda	27.40	25.00	0.70	41.50	5.80
Parmesan	25.80	35.70	1.18	29.20	5.40
Cream	34.90	7.50	0.08	53.70	4.60
Cottage	4.50	13.60	0.06	79.00	5.00
Colby	32.10	23.80	0.68	38.20	5.1-5.4

Adopted form (Kosikowski and Mistry, 1977; Scott, 1986)

*g/100g

Table 3. Advantages and sources of some ingredients used in processed cheese.

Ingredient	Advantages	Example
Lactose	Cheap filler, be less than 17% soluble in water to avoid the mallard or crystallization reactions	Whey powder, lactose powder, skim milk powder, evaporated milk, and liquid whey.
Fat	Provide the composition, texture, and meltability properties that are needed	Cream, butter, and anhydrous milk fat.
Protein	Compositional specification, texture, and meltability qualities; help produce a physiochemically stable product.	Milk protein isolates and concentrates, micellar casein powder, skim milk powder, acid casein, rennet casein, casein, whey protein isolates and concentrates, and milk protein. Hydrolysates, ultrafiltered milk
Stabilizers	Generate stable products that are resistant to chemical changes as well as provide the desired texture and meltability characteristics	Emulsifying salt, hydrocolloids, gums.

Adopted from (Guinee, 2009)

Table 4. Amount of natural cheese and functional properties of various processed cheese.

Type of Processed cheese	% Natural cheese	Functionality	Reference
Block	-70- 75% mild cheese - 25-30% semi-mature	- Less content of elastic	(Thomas, 1973)
Slices	- 55% young cheese - 35% medium cheese - 10% aged cheese	- High content of elastic - High intact protein (unhydrolyzed)	(Kosikowski and Mistry, 1977)
Spread	- 30% young cheese - 50% semi-mature cheese - 20% mature cheese	- Hydrolyzed protein	(Meyer, 1973)

Table 5. Effect of addition of ingredients on the physical properties of processed cheese.

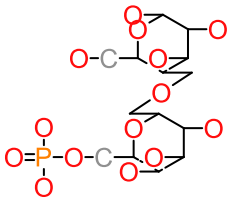
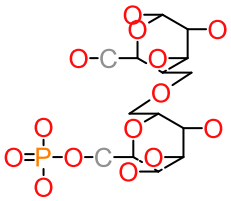
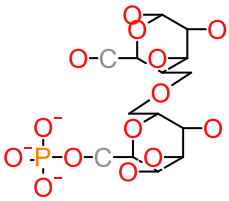
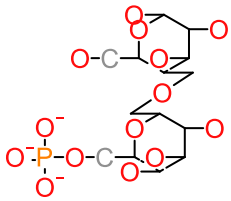
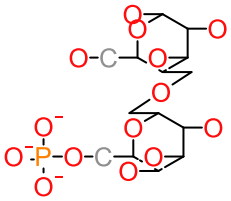
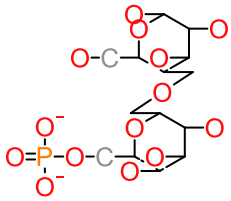
Ingredients	Viscosity	Firmness	Meltability
Fat	Decrease	Decrease	Increase
Moisture	Decrease	Decrease	Increase
Whey protein concentrate (WPC)	Increase	Increase	Decrease
pH	Increase	Increase	Decrease
Emulsifying salts	Increase	Increase	Decrease
Intact casein	Increase	Increase	Decrease

Table 6. Analysis of the lactose phosphate content of lactose powders by capillary electrophoresis (n = 10).

Sample	Lactose phosphate (ppm)
Regular milk	10 ± 0.2
Whey	0.7 ± 0.1
Edible grade lactose	368 ± 6
Pharmaceutical grade β-lactose	250 ± 4.8
Pharmaceutical grade α-lactose 1 Batch 2	252 ± 3.9
Pharmaceutical grade α-lactose 1 Batch 1	204 ± 3.7
Pharmaceutical grade α-lactose	144 ± 2.5
Ultra-pure lactose	No detectable peak

ppm or mg of lactose phosphate per kg of lactose
(Lifran, 2007; Thum et al., 2015)

Table 7. Ionic state of lactose phosphate as a function of pH.

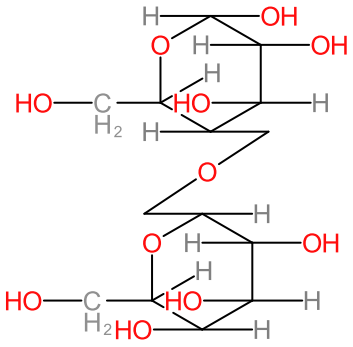
pH <1	pKa_1 =1	pH= 3 to 4	pKa_2 =6	pH> 8
$C_{14}H_{27}O_{14}P$	- 50% as $C_{14}H_{27}O_{14}P$	$C_{14}H_{26}O_{14}P$	-50% as $C_{14}H_{26}O_{14}P$	$C_{14}H_{25}O_{14}P$
				
	-50% as $C_{14}H_{26}O_{14}P$		-50% as $C_{14}H_{25}O_{14}P$	
				

Adapted from (Lifran, 2007)

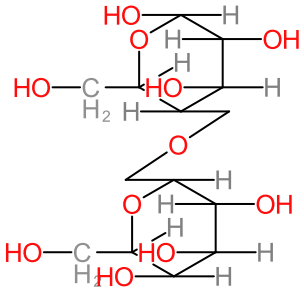
Table 8. Proximate composition of different types of milk powder. Adapted from Tsermoula et al. (2021)

Component	Lactose	Protein	Ash	Total solids	Calcium	Phosphorus	Sodium
	w/w %				mg 100 g^{-1}		
Lactose	98.1	0.24 ± 0.02	0.20	98.5 ± 0.32	58	34	31
Milk permeate powder	84.6	2.98 ± 0.05	7.48 ± 0.12	95.1 ± 0.54	744	839	972
Skim milk powder	51.4	36.6 ± 0.42	7.79 ± 0.13	95.8 ± 0.35	1289	1069	345
Milk powder isolate	1.75	87.5 ± 0.42	6.25 ± 0.11	95.5 ± 0.80	2113	1287	45

Figures



(a) α-lactose



(b) β-lactose

Figure 1. Chemical structure of (a) α-lactose and (b) β-lactose.

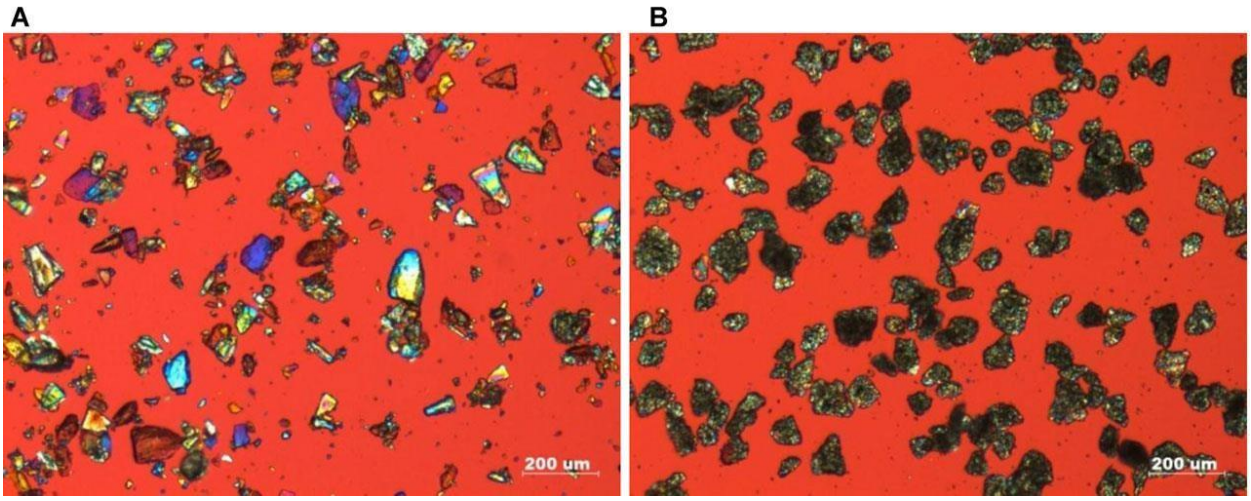
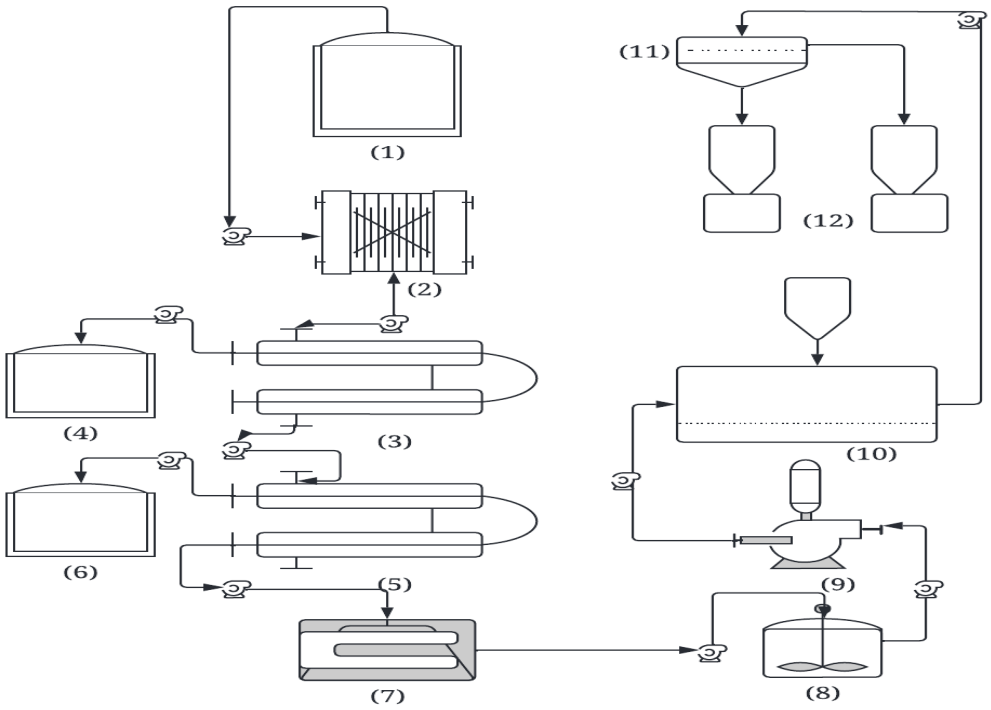


Figure 2. Microscopic images of crystal structure for (A) α-lactose crystals and (B) β-lactose Crystals (Wong and Hartel, 2014).



Whey tank	(1)	Evaporation	(7)
Pasteurization	(2)	Crystallization	(8)
Ultrafiltration	(3)	Decanter centrifuge	(9)
Retentate tank	(4)	Fluidized bed drier	(10)
Membrane concentration	(5)	Screen	(11)
Permeate tank	(6)	Baggers	(12)

Figure 3. Schematic representation of the main manufacturing steps of edible lactose manufacture from whey (Durham, 2000).

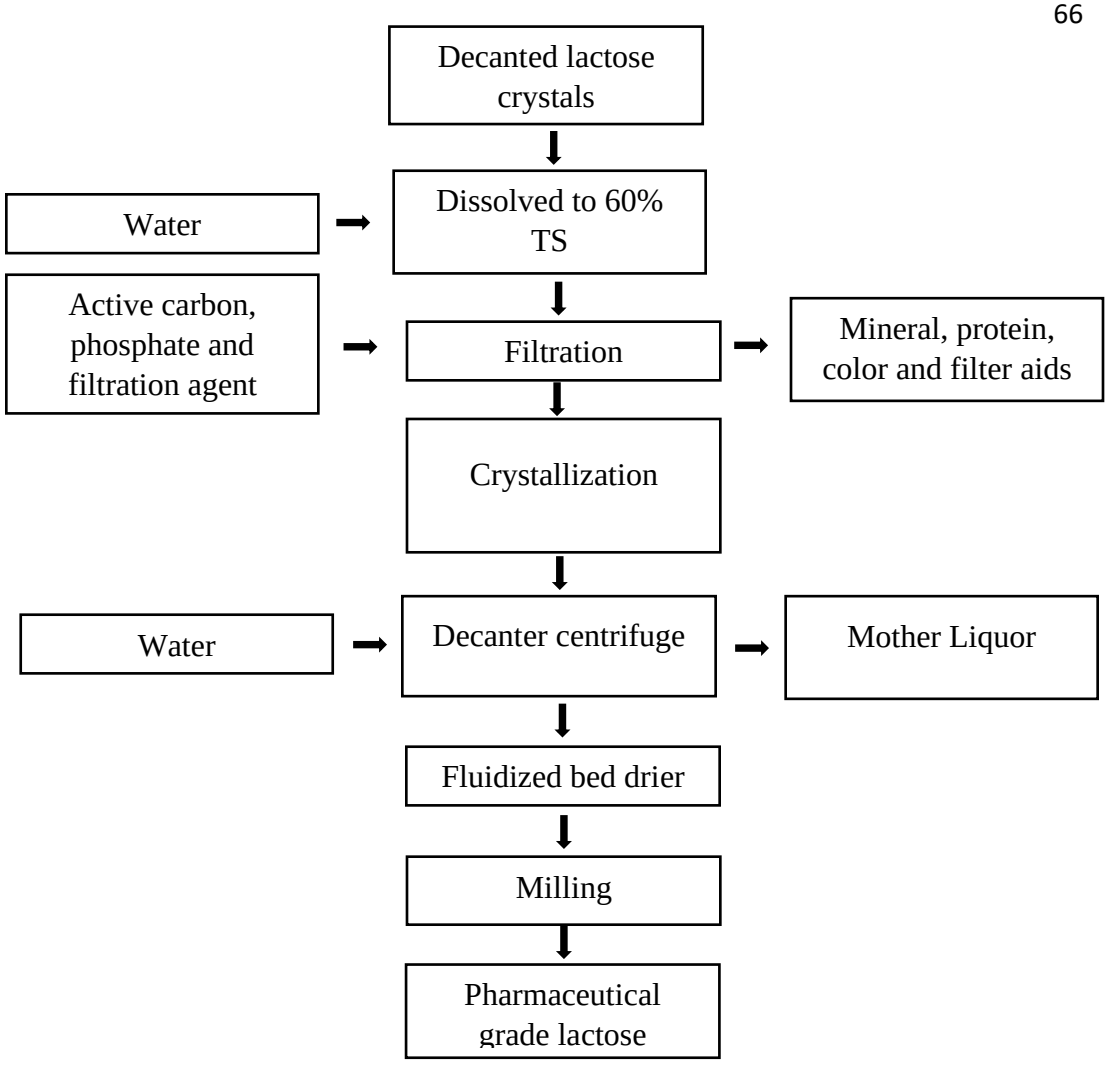
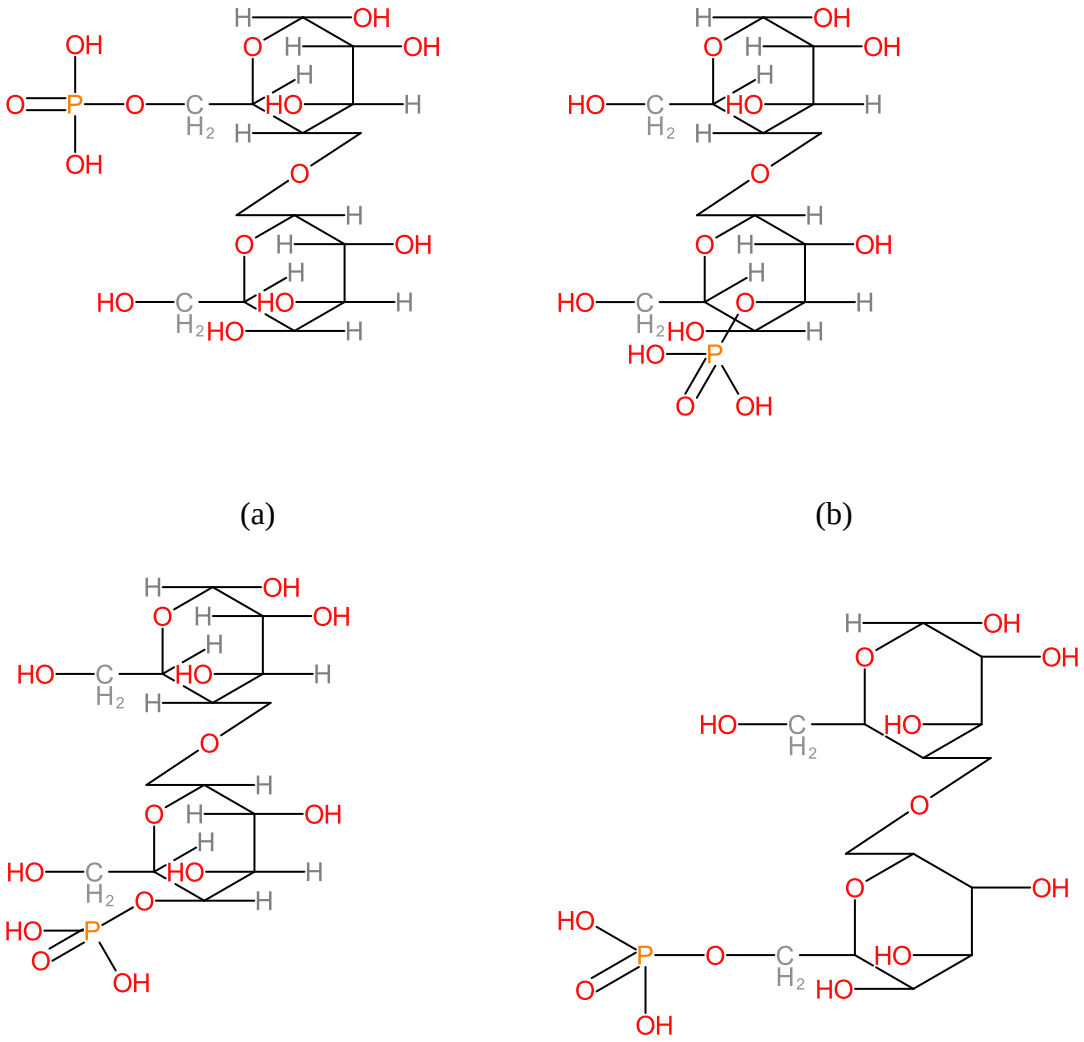


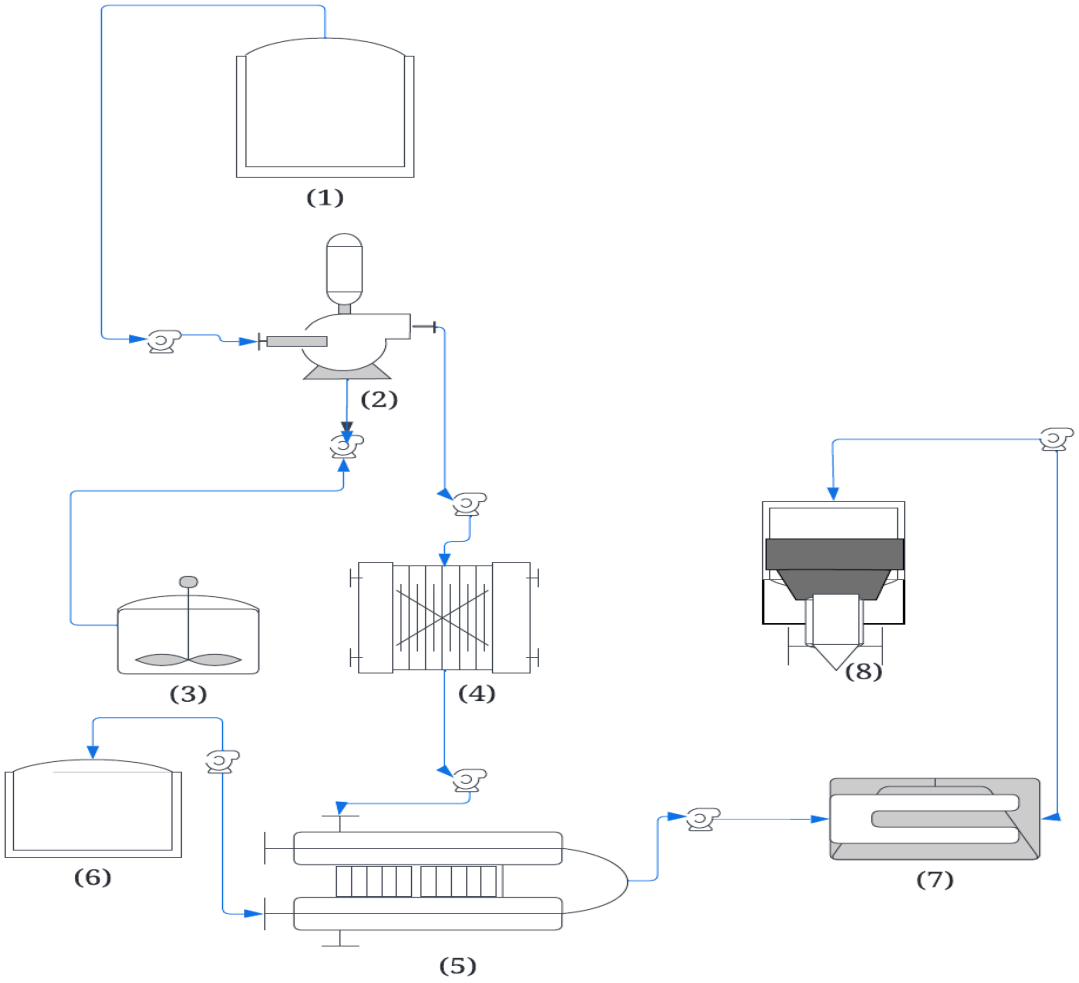
Figure 4. Process of pharmaceutical grade lactose manufacture from edible grade lactose (Pritzwald-Stegmann, 1986).



(a) (b)

(c) (d)

Figure 5. Monophosphate is placed on (a) the glucose moiety's carbon 6 on galactose moiety's carbon (b) 3 on galactose moiety's carbon, (c) 4 on galactose moiety's carbon, and (d) 6 on galactose moiety's carbon.



Raw milk tank	(1)	Centrifuge	(2)
Cream tank	(3)	Pasteurization	(4)
Membrane concentration (ultra-filtration)	(5)	Retentate tank	(6)
Evaporation or reverse osmosis filtration	(7)	Dryer	(8)

Figure 6. Schematic representation of the main manufacturing steps of milk permeate Powder (MPP).

CHAPTER III: PHOSPHORYLATION OF LACTOSE IN α-LACTOSE MONOHYDRATE AND MILK PERMEATE USING A NOVEL PROCESS

Abstract

Lactose is the primary carbohydrate in most mammals' milk, commonly known as milk sugar. Milk permeate is a byproduct of fractionating milk through membrane filtrations. It contains lactose and minerals that are used to make lactose commercially. Sugar phosphorylation is a technique used to alter the characteristics of sugar. Phosphorylation has numerous applications for developing dairy and food products. Lactose-6-phosphate (LP) is an organic compound that switches the hydrogen on lactose by monophosphate. Lactose and LP are structurally identical. The objectives of this study were to develop a method to phosphorylate lactose and milk permeate powder (MPP) at specific concentrations that include pH, temperature, and time. The first solution (LaP1) was prepared by mixing 1 mol of α-lactose with 0.5 mol of sodium cyclo-triphosphate. The second solution (LaP2) was prepared by mixing MPP and sodium phosphate dibasic in distilled water at a concentration of 28.32 and 1.41%, respectively. The pH of both solutions was adjusted using 40% sodium hydroxide to get a pH of 12. The solutions were stirred for 3 d at room temperature and then stored at 4°C for 24 h. The solutions were separated into two layers, and the bottom layers were decolorization using activated carbon. The LaP1 and LaP2 solutions were freeze dried. A mass spectrometry (MS) was used to define LP in both solutions. Two control samples were utilized, which were α-lactose monohydrate (control 1) and MPP (control 2). The molecule mass of lactose ranged from 341.46 to 341.80 Da, but it ranged from 421.23 to 422.0 Da for LP. The amount of lactose was dropped in LaP1 (15.58%) and LaP2 (12.20%) compared to

control 1 (69.32%) and control 2 (24.64%). However, the level of LP was increased in LaP1 (60.74%) and LaP2 (8.65%), while it was 0.89 and 5.53% for control 1 and control 2, respectively. We conclude that lactose and milk permeate can be phosphorylated, and MS can be used to detect lactose and LP.

Keywords: Phosphorylation; Lactose; Milk permeate; Mass spectrometry; Lactose-6-phosphate

Introduction

Lactose is a disaccharide carbohydrate made up of glucose and galactose molecules, which are linked by β-1, 4 glycosidic bonds (Figure 1). Lactose is the mean carbohydrate and only known source in milk (0-10% w/w) (Fox, 2009). Milk permeate is a byproduct of milk fractionation through membrane filtrations. Milk permeate contains lactose and minerals that are used to purify and fractionate lactose further. Lactose is widely used in food and pharmaceutical industries because it is a cheap ingredient, source of low sweetness, and filler (Lifran et al., 2000). Lactose is also significant for its economic worth and contributions to the sensory and functional aspects of dairy products. Lactose is a cheap dairy ingredient that could increase the profit of dairy products, but it could also increase some chemical reactions in these products.

Sugar phosphorylation is a process used to change the properties of sugar. It has many applications that can be used to develop dairy and food products. Increasing the hydrophilicity of starch through phosphorylation is a common chemical modification method that produces products useful for the food, paper, glue, textile, and pharmaceutical industries (Solarek, 1986; Chiu and Solarek, 2009). Previous studies'

findings demonstrated that the phosphorylation approach had been successfully used to enhance the properties of mung bean, rice, corn, wheat, sago, and other starches (Lim and Seib, 1993; Muhammad et al., 2000; Lin et al., 2009; Nathania et al., 2017). The pH plays a significant impact in altering the ratio of monoester to diester linkages during phosphorylation (Muhammad et al., 2000). Several researchers have examined the phosphorylation of lactose with phosphate (Inoue et al., 2002; Lifran, 2007).

Lactose-6-phosphate (LP) is naturally present in milk and milk products with low concentrations (Lifran, 2007; Thum et al., 2015). LP was initially identified in bovine milk (Cumar et al., 1965) and was recently identified in caprine milk (Albrecht et al., 2014). It presents in low concentrations, and it is hard to be fractionated and purified due to the similar structure to lactose, LP is an organic molecule that replaces lactose's hydrogen with monophosphate. A phosphate group is connected to the lactose galactose portion of most LP molecules (Figure 2). Approximately 90% of LP is coupled to galactose in pharmaceutical-grade lactose, while 10% is bound to glucose (Breg et al., 1988). There are not many studies on the LP available, as well as its characteristics and applications in dairy products. Obtaining the optimum LP combination, finding pure lactose free of LP and other contaminants, directly analyzing LP, and investigating its features were the challenges that led to the limitations of LP investigations. Similarly, the lack of knowledge regarding the origin of this ingredient is significant. The presence of LP could limit the crystallization of pharmaceutical-grade lactose, according to Visser (1988).

A mass spectrometry (MS) is powerful equipment used to measure the mass-to-charge ratio (m/z) of molecules present in a sample. It has many applications, including

molecular weights, isotopes, abundance, elucidation of the chemical identity, and structure of molecules (Aebersold and Mann, 2003; Kostiainen et al., 2003; Lisec et al., 2006). Indeed, no LP standard is available; thus, we developed our methods. A previous study demonstrated that LP could be presented in milk and milk products, but with a low concentration, it is hard to separate, extract, and purify LP from lactose. The objectives of this study were to develop a method for phosphorylating α-lactose monohydrate (LaP1) and milk permeate powder (LaP2) as well as using MS to define lactose and LP in α-lactose monohydrate, MPP, LaP1, and LaP2.

Material and methods

Lactose-6-phosphate preparation

LP was prepared using the methodology reported by Inoue et al. (2002) with some modifications. Two different solutions were prepared using α-lactose monohydrate and milk permeate powder (MPP), as presented in Table 1. The first solution (LaP1) was prepared by mixing 1 mol of α-D- (+) lactose monohydrate ($C_{12}H_{22}O_{11}.H_2O$: Fisher chemical; L5-500) with 0.5 mol of sodium cyclo-triphosphate (Frontier Scientific; Cat # 343031). The second solution (LaP2) was prepared by mixing MPP obtained from Idaho Milk Products (Jerome, ID, USA) and sodium phosphate dibasic (DSP) (Fisher Scientific, Fair Lawn, New Jersey) in distilled water at a ratio of 28.32 and 1.41%, respectively. The pH of both solutions was adjusted using sodium hydroxide to get a pH of 12. The solutions were stirred for 3 d at room temperature and then stored at 4°C for 24 h. The solutions were separated into two layers, and the bottom layers were used in the study.

Decolorization of lactose-6-phosphate solutions

The decolorization of LaP1 and LaP2 solutions was prepared using the methodology reported by Zhang et al. (2015) with some modifications. During the phosphorylation process, the color of solutions turns dark, as shown in Figures 3 and 4. These solutions were decolorized using activated carbon (Darco G60,-100 mesh, powder, Aldrich Chemical Company). The bottom layers were diluted with distilled water with a ratio of 1:2.2 into a 1000 mL glass Erlenmeyer flask. A 25 g of activated carbon was added to 250 ml of the lower portion of solutions, mixed well, and set for 5 min at room temperature. The mixture was filtered through filter paper (Cat No 1001 125, Whatman). The mix and filtration steps were repeated seven times until having transparent (colorless) solutions. Up to 25% of the volume of the solutions might be lost during the preparation.

Freeze dryer

LaP1 and LaP2 solutions were freeze dried after decolorization using the methodology reported by Jouppila and Roos (1994) with some modifications. The LaP1 and LaP2 solutions were freeze dried using a Labconco freeze-dryer (Lyph-Lock 6, Labconco, Kansas City, KS, USA) for 72 h (temperature < −40°C; pressure < 0.1 mbar). For freeze drying, the solutions were poured into Petri dishes (15 mL in each to prepare samples) and frozen at −79°C using an ultralow freezer (NUAIRE - 85±1°C Ultralow Freezer) for 24h.

Mass spectrometry

MS was obtained using an Qtrap 5500 triple quadrupole MS (AB Sciex, Foster City, CA, USA). Detection was achieved by electrospray ionization (ESI), operating in negative ion mode, with precursor ion scans performed with an m/z range of 300-450. Nitrogen (20 psi) was used as the curtain gas. The ion source was operated at -4500 volts (V), a temperature of 300°C, and a pressure of 14 psi for nebulizer (GS1) gas. Default settings were used for the entrance potential of the collision cell (-10V), declustering potential (-90V), collision energy (-38V), and collision cell exit potential (-19V). Each sample was prepared in a concentration of 100 ug/mL (by weight) in water. All samples were filtered with a 4 mm, 0.2 um nylon syringe filter before analysis. Three different batches of LaP1 and LaP2 were used in this study. Two samples were applied as controls. Control 1 and control 2 were used α-lactose monohydrate and MPP, respectively.

Compositional analyses

The final LaP1 and LaP2 solutions were analyzed for moisture using a forced draft oven (AOAC, 2000, method 990.20; 33.2.44), and pH was determined using the Hanna pH meter **(**Hannah Edge Blu, Woonsocket, RI 02895**).**

Statistical Analysis

Statistical analysis was performed to study the TS, pH, and relative abundances of lactose and LP in α-lactose monohydrate, milk permeate, LaP1, and LaP2. One way ANOVA test was done using R software (R ×64-3.3.3, R Foundation for Statistical

Computing). Mean separation was done using the least significant difference (LSD) test at $P< 0.05$.

Results and discussions

Composition of phosphorylation solutions

Table 2 shows the TS and pH of LaP1 solutions. The TS of the down layer of LaP1 was 27.92%. When this was diluted with distilled water with a ratio of 1:2.2 resulted in 12.61% TS. The TS of LaP1 after the decolorization process was 6.03%. The TS was significantly decreased ($P<0.05$) after the decolorization process. The loss of TS is related to removing the organic compounds from the solution by the adsorbent technique of activated carbon. It is not entirely clear how organic molecules are adsorbed on activated carbon in an aqueous media. Weber Jr and Morris (1963) theorized that intraparticle diffusion is the critical step in the adsorption of several organic compounds on carbon.

In a turbulent environment, several mechanisms are involved in the adsorption of organic compounds on activated carbon. The pH of the down layer of LaP1 before dilution, after dilution, and decolorization was 11.70, 11.70, and 10.86, respectively. A significant difference ($P<0.05$) was detected between the pH of the diluted solution and the decolorization solution. The pH of solutions decreased ($P<0.05$) after the decolorization process. The pH of the solutions critically affects the activated carbon behavior. A study on sugar syrup shows that the pH between 4.5 to 6.7 was decolorized easily compared to the pH of 2.2 and 10.2 (Mudoga et al., 2008). As the solutions were in alkali conditions, this could give activated carbon treatment a poor efficiency. This could

be related to the hydroxide ions on the solutions, which are competitive ions to color removal (Obeid et al., 2013). The pH adjustment of solutions before decolorization is recommended to improve the efficiency of activated carbon (Bansal and Goyal, 2005). However, this adjustment of pH could affect the structure of LP.

Table 3 presents the TS and pH of the LaP2 solutions. The TS of the down layer, diluted solution, and decolorization solution of LaP2 were 30.12, 8.8, and 4,93%, respectively. A significant difference ($P<0.05$) was detected between diluted and decolorized solutions. Because of its non-polar nature, activated carbon is notably well recognized for adsorbing organic compounds (Bernardo et al., 2006). It is effective in adsorbing different types of chemicals. This led to a decrease in the TS of solutions after being treated with the activated carbon. Similar results have found that TS was decreased for beet sugar after being treated with activated carbon (Harris, 1942). The pH of the down layer, diluted, and decolorization solution of LaP2 were 11.66, 11.54, and 10.37, respectively. The pH of LaP2 solutions was significantly decreased ($P<0.05$) after the decolorization process. This is related to removing organic compounds by adsorption techniques, leading to a decreased solution pH (Cendekia et al., 2022). Similar results were found by Lubis et al. (2020). They purified water using activated carbon from natural sources. They found a decrease in water pH after being treated with activated carbon.

Qualitative determination

MS was used to detect lactose and LP in α-lactose monohydrate, MPP, LaP1, and LaP2. MS is superior in terms of accuracy and precision to other methods for measuring

molecular masses of sugars in terms of actual molecular masses (Smith et al., 1990). Within the studied molecular mass range (300-450 m/z), the corresponding spectra exhibit the theoretical molecular masses for lactose and LP, which are 342.3 and 422.28 Da, respectively. LP identification relies heavily on the difference between lactose and LP molecular masses.

Table 4 presents the mean molecular masses of lactose and LP for α-lactose monohydrate, MMP, LaP1, and LaP2. The lactose molecule mass of treatments ranged from 341.46 to 341.80 m/z, which we assume is related to lactose. In contrast, LP molecule mass of treatments ranged from 421.23 to 422.05 Da, which might be related to LP. The mass differences between experimental and theoretical masses for lactose and LP were 0.84 and 1.05 Da, respectively. Similar results were found in Chen et al. (2004), who compared the experimental and theoretical molecular masses of major whey protein fractions in goat and cow milk using MS. They found differences between the experimental and theoretical molecular masses between the samples.

Table 5 presents the relative abundance of lactose for control 1, control 2, LaP1, and LaP2, which were 69.32, 24.64, 15.58, and 12.20%, respectively. The ANOVA with MS and P-values for lactose and LP of control 1, control 2, LaP1, and LaP2 is shown in Table 6. The amount of lactose significantly dropped ($P<0.05$) for control 1 and LaP1. Moreover, it was slightly decreased ($P>0.05$) between control 2 and LaP2, which is expected. The amount of lactose is reduced due to the chemical reaction between the monosaccharides and hydrolyzed disaccharides with sodium hydroxide (Zheng et al., 2015). In addition, the degradation of lactose under alkalis conditions has been noted (Lewkowski, 2001). A study done on hemicellulose sugar shows that the alkali conditions

release acetyl and acidic groups and organic acids that lead to lower pH (Nikzad et al., 2014). Moreover, the Maillard reaction could occur when the carbonyl compounds in sugar combine with the amino acids to generate an unstable Schiff base. Then, via double bond migration and rearrangement activities of the Schiff base, the reduction efficiency may increase during the development of relatively stable Amadori or Heyns rearrangements (Cristina De Oliveira et al. 2016). The intermediate step of the Maillard reaction entails Amadori product degradation, notably sugar dehydration and deamination, Strecker degradation, and sugar fragmentation (Xiang et al., 2021). Moreover, the lactose could contain lactulose, lactitol, gluconic, other acids, lactosylurea, N-substituted amino sugars, and polymers, and other reactions include esterification and acetylation could occur under the alkali condition (Holsinger, 1997).

The relative abundance of LP was 0.89, 5.53, 60.74, and 8.65% for control 1, control 2, LaP1, and LaP2, respectively. LaP1 had a higher ($P<0.05$) LP level than other treatments. Similar results were found in Inoue et al. (2002), who phosphorylated lactose to obtain 33.0% LP. However, there was a slight increase in LP for LaP2 compared to control 2. At the same time, there was a slight decrease in the lactose level. LP was detected in all treatments with various levels. Previous studies have found that the LP was presented at lower concentrations in lactose and whey, that was 368.0 and 0.70 ppm, respectively (Lifran, 2007; Thum et al., 2015).

Figures 5 and 6 show the chromatogram of α-lactose monohydrate and LaP1. The results reveal main peaks at 341.46 and 421.23 m/z for lactose and LP, respectively. Figures 7 and 8 present the mass spectra of MMP and LaP2, respectively. For MMP, lactose (341.6, 30.14 m/z) was observed, whereas for LP (421.2, 3.97 m/z) was

observed. Lactose and LP were at 341.6 m/z (11.93%) and 421.3 m/z (11.31%) in LaP2, respectively. Additionally, a characteristic fragment for lactose and LP at 341.53 and 421.38 m/z are visible in each spectrum. The results demonstrate that lactose and LP are the main compounds in LaP1 and LaP2, and they are present in approximately equal amounts in LaP2, while LP was higher than 15x lactose in LaP1. The difference in LP in LaP1 and LaP2 could be due to the difference in lactose concentration that was started with. The relative abundance of lactose in α-lactose monohydrate was 2.81x higher compared to MPP.

Conclusions

LP was prepared successfully using α-lactose monohydrate and MPP. The pH and TS of LaP1 and LaP2 were significantly decreased after the decolorization process. MS was successfully used to determine the amount of lactose and LP. The remaining lactose in LaP1 and LaP2 decreased by about 22.47, and 49.51%, respectively. LP was increased after the phosphorylation process in LaP1 and LaP2. When comparing LaP1 and LaP2, we found a higher amount of LP in LaP1. This study concluded that the relative abundance of LP in MPP was higher than α-lactose monohydrate.

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Tables

Table 1. Mean (n=3) of phosphorylation lactose (LaP1) and phosphorylation milk permeate (LaP2) formulations (g $100g^{-1}$).

	Ingredients					
Treatments	**α-lactose monohydrate**	**Milk permeate powder**	**Sodium phosphate Dibasic**	**Sodium cyclo-triphosphate**	**NaOH (40%)**	**Water**
Phosphorylation lactose (LaP1)	22.65	-	-	9.59	5.01	62.78
Phosphorylation milk permeate (LaP2)	-	28.32	1.41	-	13.54	56.65

Table 2. Mean (n=3) composition of phosphorylation lactose (LaP1).

Treatments[1]	Total solids (%)	pH
Phosphorylation lactose (LaP1)	27.92[a]	11.70[a]
Diluted solution	12.61[b]	11.72[a]
Decolorization solution	6.03[c]	10.86[b]

[1]Treatments: Phosphorylation lactose (LaP1) = The bottom layer of LaP1 after the phosphorylation process; diluted solution= The bottom layer of LaP1 after the phosphorylation process and diluted with distilled water with a ratio of 1:2.2; and decolorization solution = LaP1 after washed 7 times with activated carbon

[a-c] Means in the same row not sharing a common superscript are different at $P<0.05$

Table 3. Mean (n=3) composition of phosphorylation milk permeate (LaP2).

Treatments[1]	Total solids (%)	pH
Phosphorylation milk permeate (LaP2)	30.12[a]	11.66[a]
Diluted solution	8.83[b]	11.54[a]
Decolorization solution	4.93[c]	10.37[b]

[1]Treatments: Phosphorylation milk permeate (LaP2) = The bottom layer of LaP2 after the phosphorylation process; diluted solution= The bottom layer of LaP2 after the phosphorylation process and diluted with distilled water with a ratio of 1:2.2; and decolorization solution = LaP2 after washed 7 times with activated carbon

[a-c] Means in the same row not sharing a common superscript are different at $P<0.05$

Table 4. Mean (n=3) mass spectrometry (MS) data on the experimental molecular mass and formula of lactose and lactose-6-phosphate (LP) for α-lactose monohydrate, milk permeate powder (MPP), phosphorylation lactose (LaP1), and milk permeate (LaP2).

Treatments[1]	Lactose		Lactose-6-phosphate	
	Molecular mass (Da) ± SD	**Formula**	**Molecular mass (Da) ± SD**	**Formula**
Control 1	341.80 ± 0.43	$C_{12}H_{22}O_{11}$	422.05 ± 1.06	$C_{12}H_{23}O_{14}P$
Control 2	341.53 ± 0.11		421.23 ± 0.05	
LaP1	341.46 ± 0.12		421.23 ± 0.15	
LaP2	341.53 ± 0.11		421.38 ± 0.41	

Treatments[1]: Control 1 = α-lactose monohydrate without any treatment; Control 2= Milk permeate powder without any treatment; LaP1= The powder of phosphorylation lactose; LaP2= The powder of phosphorylation milk permeate

Table 5. Mean (n=3) relative abundance of lactose and lactose-6-phosphate (LP) for α-lactose monohydrate, milk permeate powder (MPP), phosphorylation lactose (LaP1), and milk permeate (LaP2) using a mass spectrometry (MS).

Treatments[1]	**Lactose** (Relative abundance % ± SD)	**Lactose-6-phosphate** (Relative abundance % ± SD)
Control 1	69.32 ± 16.87[a]	0.89 ± 0.16[a]
Control 2	24.64 ± 8.46[b]	5.53 ± 1.05[a]
LaP1	15.58 ± 9.44[b]	60.74 ± 38.89[b]
LaP2	12.20 ± 2.11[b]	8.65 ± 2.62[a]

Treatments[1]: Control 1 = α-lactose monohydrate without any treatment; Control 2= Milk permeate powder without any treatment; LaP1= The powder of phosphorylation lactose; LaP2= The powder of phosphorylation milk permeate

[a-b] Means in the same row not sharing a common superscript are different at $P<0.05$

Table 6. Mean squares and P-values of lactose and Lactose-6-phosphate (LP) with either α-lactose monohydrate, milk permeate powder, phosphorylation lactose (LaP1), or phosphorylation milk permeate (LaP2).

Factor	**df**	**Lactose**	**Lactose-6-phosphate**
Replication	2	6.95 (0.95)	366.91 (0.43)
Treatment	3	2098.41 (<0.05) **	2358.61 (<0.05) *
Error	6	140.06	384.64

* Statistically significant at $P < 0.05$

** Statistically significant at $P < 0.05$

Figures

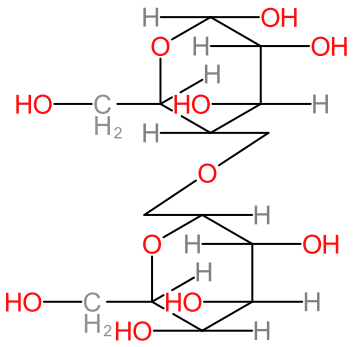


Figure 1. Chemical structure of α-lactose

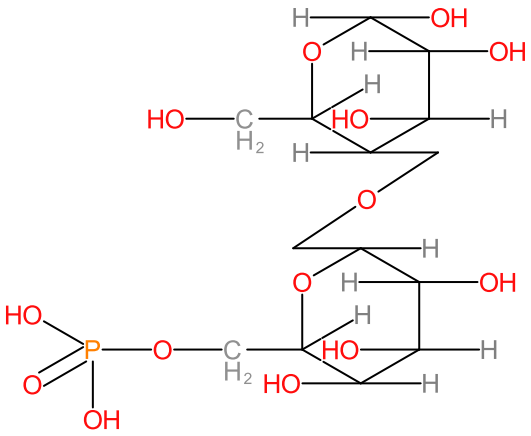
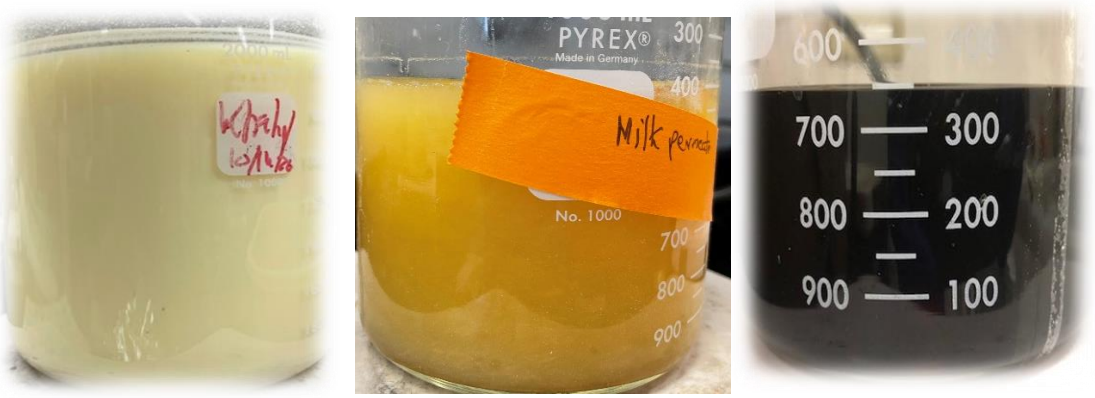


Figure 2. Chemical structure of lactose-6-phosphate (phosphate on galactose moiety on carbon number 6)

(a) (b) (c)

Figure 3. The color of phosphorylation lactose (LaP1): (a) LaP1 in the first 24h, (b) LaP1 after 48h, (c) LaP1 after 72 h.



(d) (e) (f)

Figure 4. The color of phosphorylation milk permeate (LaP2): (d) LaP2 in the first 24h, (e) LaP2 after 48h, and (f) LaP2 after 72 h.

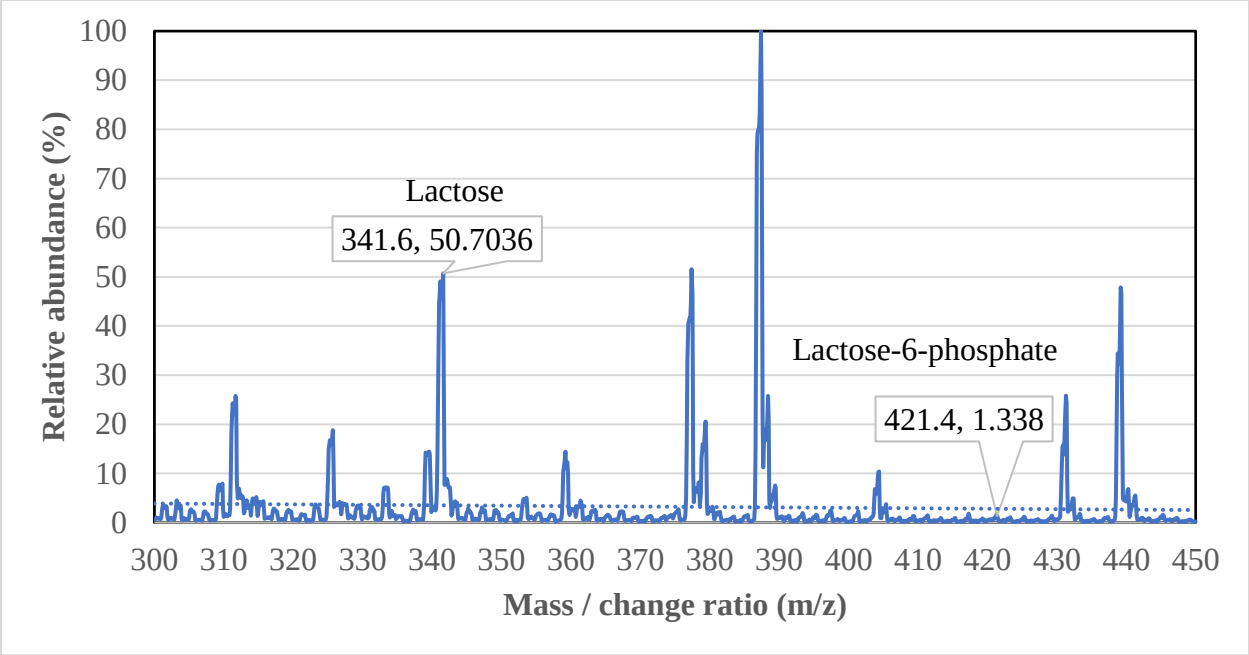


Figure 5. Mass spectrum of α-lactose monohydrate.

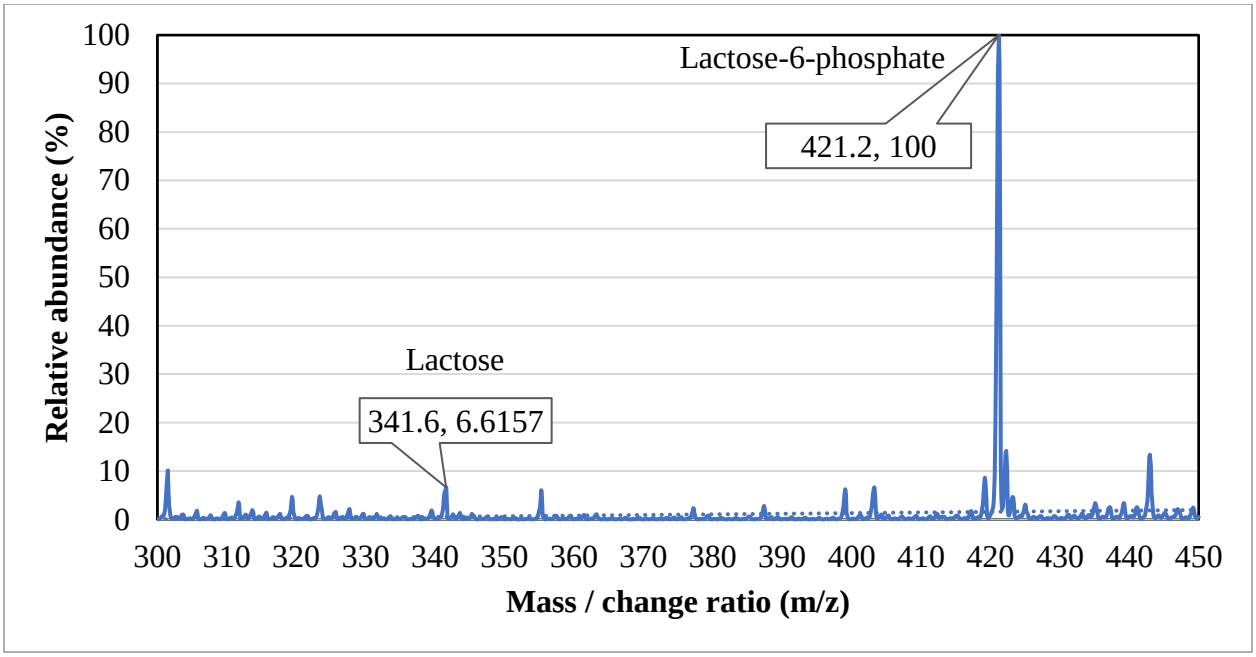


Figure 6. Mass spectrum of lactose-6-phosphate (LaP1) made by α-lactose monohydrate.

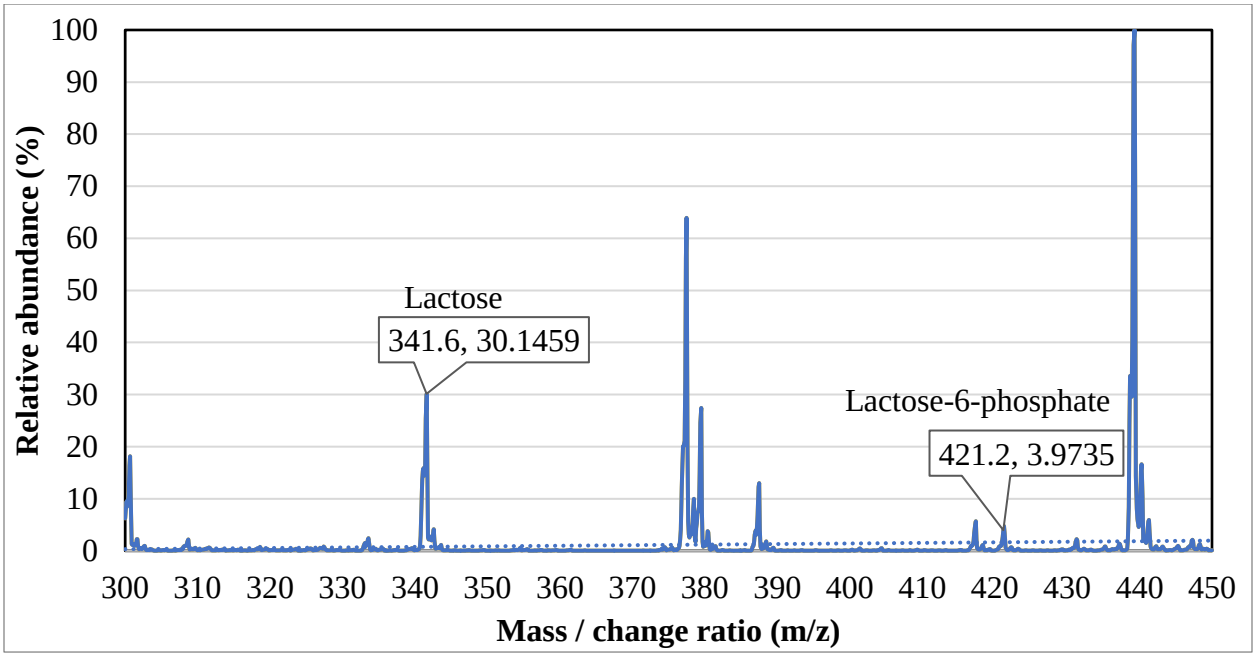


Figure 7. Mass spectrum of milk permeate powder (MPP).

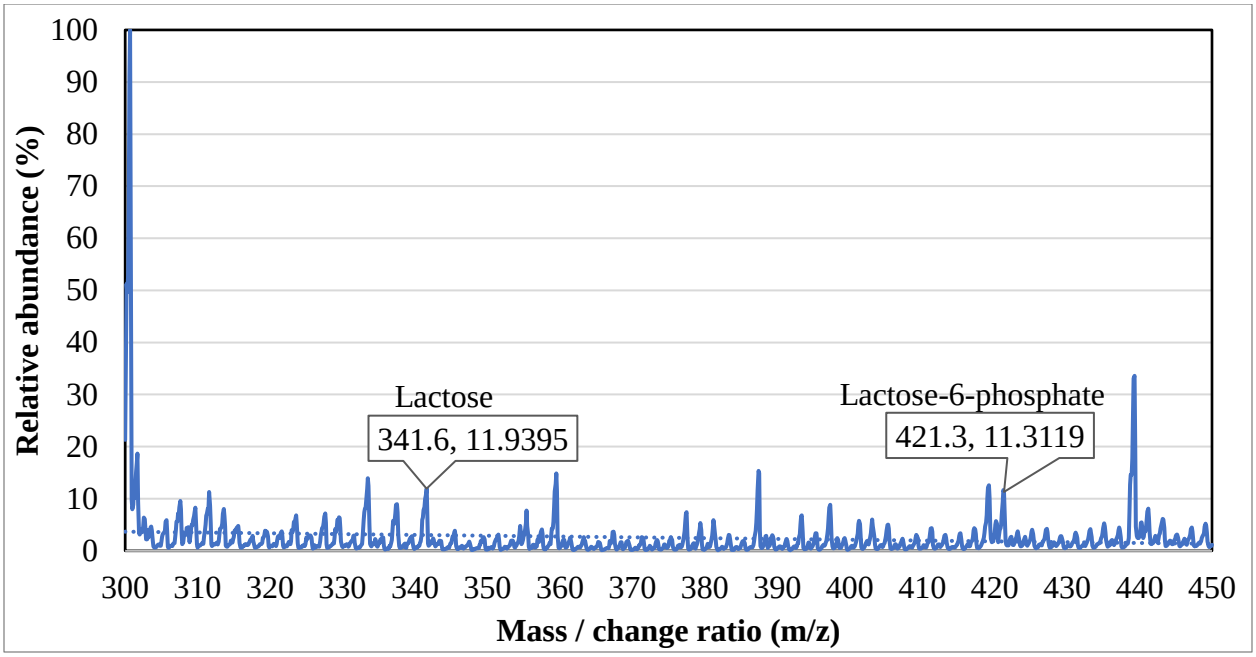


Figure 8. Mass spectrum of Lactose-6-phosphate made by milk permeate powder (LaP2).

CHAPTER IV: DECOLORIZATION OF LACTOSE-6-PHOSPHATE SOLUTIONS USING ACTIVATED CARBON

Abstract

Sugar phosphorylation has many applications that can be used to develop dairy and food products. During the phosphorylation process, the color of the solution turns into dark color. The dark color causes many challenges and limitations in using phosphorylation products. The dark color could cause unpleasant color changes on the products, so it is significant to remove that color. Activated carbon has been utilized for decades to remove the dark color and improve the appearance of solutions such as syrup sugar and wastewater. This methodology is cheap method and environmentally friendly. The objectives of this study were to develop a method to phosphorylate α-lactose monohydrate and milk permeate and to remove the dark color of solutions. The compositional characteristics of the solution, such as pH, total solids, and color parameters (L*- lightness, a*- redness, and b*- yellowness) were examined at different stages (seven stages) of washing the solutions. α-lactose monohydrate and MPP solutions were diluted with distilled water with a ratio of 1:2.2. Activated carbon was mixed with the solutions and left for 5 min at room temperature. Subsequently, the solutions were filtered. These steps were repeated seven times until having transparent (colorless) solutions. The experiment was repeated using three different batches of lactose and milk permeate solutions. Both solutions' pH and total solids decreased with increasing the number of washings with activated carbon. The International Commission on Illumination (CIE) L*a*b* scale was studied. The L* of the initial solutions was lower than the final solutions. However, the a* and b* of the initial solutions were higher than

the final solutions. The total color difference (ΔE) was calculated for both solutions. The ΔE was decreased with increasing the number of washings with activated carbon in both solutions. We conclude that activated carbon can be used to remove the dark color that results from the phosphorylation process.

Keywords: Phosphorylation; Lactose; Milk permeate; Activated carbon; Lactose-6-phosphate

Introduction

Sugar phosphorylation has many applications that can be used to develop dairy and food products. Increasing the hydrophilicity of starch through phosphorylation is a common chemical modification method that produces products useful for the food, paper, glue, textile, and pharmaceutical industries (Solarek, 1986; Chiu and Solarek, 2009). Previous studies' findings demonstrated that the phosphorylation approach had been successfully used to enhance the attributes of mung bean, rice, corn, wheat, sago, and other starches (Lim and Seib, 1993; Muhammad et al., 2000; Lin et al., 2009; Nathania et al., 2017).

Lactose, a disaccharide carbohydrate ($C_{12}H_{22}O_{11}$), is the mean carbohydrate in milk. Whey permeate, a byproduct of cheese and fractionation casein from skim milk, is used to make lactose commercially. It is widely used in food and pharmaceutical industries because it is a cheap ingredient, source of low sweetness, and filler (Lifran et al., 2000). Lactose is also significant for its economic worth and contributions to the sensory and functional aspects of dairy products. Lactose is a cheap dairy ingredient that could increase the profit of dairy products, but it could increase some chemical reactions

in these products. High concentrations of lactose in those products could result in lactose crystal formation and a non-enzymatic browning reaction.

Lactose-6-phosphate (LP) is naturally present in milk and milk products with low concentrations (Lifran et al., 2007; Thum et al., 2015). LP was initially identified in bovine milk (Cumar et al., 1965) and caprine milk (Albrecht et al., 2014) in low concentrations. Due to the similar structure of LP to lactose, it is hard to be fractionated and purified. LP is an organic molecule that replaces lactose's hydrogen with monophosphate. A phosphate group is connected to the lactose galactose portion of most LP molecules. Approximately 90% of LP is linked to galactose in pharmaceutical-grade lactose, while 10% is bound to glucose (Breg et al., 1988).

LP could be made using lactose and sodium cyclo-triphosphate at alkali conditions using sodium hydroxide (Inoue et al., 2002). The reaction causes color changes and byproducts such as carbon dioxide, carbon, and water. Under the alkali condition, the lactose could contain lactulose, lactitol, gluconic, other acids, lactosylurea, N-substituted amino sugars, polymers, and other reactions esterification and acetylation (Holsinger, 1997). The amount of sugar could be reduced due to the chemical reaction between the monosaccharides and hydrolyzed disaccharides with sodium hydroxide (Zheng et al., 2015). In addition, the degradation of lactose under alkalis conditions has been noted (Lewkowski, 2001). A study done on hemicellulose sugar shows that the alkali conditions release acetyl, acidic groups, and organic acids that lead to lower pH (Nikzad et al., 2014).

Moreover, the Maillard reaction could occur when the carbonyl compounds in sugar combine with the amino acids to generate an unstable Schiff base. Then, via double

bond migration and rearrangement activities of the Schiff base, the reduction efficiency may be increased during the development of relatively stable Amadori or Heyns rearrangements (Cristina De Oliveira et al. 2016). The intermediate step of the Maillard reaction entails Amadori product degradation, notably sugar dehydration and deamination, Strecker degradation, and sugar fragmentation (Xiang et al., 2021).

The color produced during LP production is complicated to determine due to the chemical structure of some of these coloring materials. The most important colored substances that form during sugar processing fall into three categories: (a) melanins, (b) melanoidins, and (c) caramels (Kearsley and Dziedzic, 1995). Different methodologies have been examined to decolorize solutions, including microorganism-mediated treatment (Dahiya et al., 2001; Ghosh and Bhattacharyya, 2002; Adikane et al., 2006) and physicochemical methods (Vittadini et al., 2003; Mane et al., 2006). Adsorption on activated carbon is a popular physicochemical treatment method for color removal due to its large surface area, microporous structure, high adsorption capacity, and high degree of surface reactivity (Satyawali and Balakrishnan, 2007). Because of its unique properties, the usage of activated carbon has been expanded to include decolorization, gas separation and polluted air treatment, heavy metal recovery and food processing with no hazard, and wastewater treatment (Garrido et al., 1992; Bernardo et al., 1997; Abdulkarim et al., 2002; Samsuri et al., 2014; Yorgun et al., 2016; Kosheleva et al., 2019).

This work aimed to develop a method to phosphoryl α-lactose monohydrate (LaP1) and phosphorylating milk permeate powder (LaP2) and investigate the removal of color-causing components from LP solutions by activated carbon adsorption, as well as measure the colored products' using the International Commission on Illumination (CIE)

L*a*b* values from colorimetry. Also, the effect of washing with activated carbon on the pH and total solids (TS) at different stages was studied.

Materials and methods

Lactose-6-phosphate preparation

LP was prepared using the methodology reported by Inoue et al. (2002) with some modifications. Two different solutions were prepared using lactose and milk permeate powder (MPP). The first solution (LaP1) was prepared by mixing 1 mol of α-D- (+) lactose monohydrate ($C_{12}H_{22}O_{11}.H_2O$: Fisher chemical; L5-500) with 0.5 mol of sodium cyclo-triphosphate (Frontier Scientific; Cat # 343031). The second solution (LaP2) was prepared by mixing MPP obtained from Idaho Milk Products (Jerome, ID, USA) and sodium phosphate dibasic (DSP) (Fisher Scientific, Fair Lawn, New Jersey) in distilled water at the ratio of 28.32 and 1.41%, respectively. The pH of both solutions was adjusted using sodium hydroxide to get a pH of 12. The solution was stirred for 3 d at room temperature and then stored at 4°C for 24 h. The solutions were separated into two layers, and the bottom layers were used in the study.

Decolorization of lactose-6-phosphate solution

The decolorization of LaP1 and LaP2 solutions was prepared using the methodology reported by Zhang et al. (2015) with some modifications. These solutions were decolorized using activated carbon (Darco G60,-100 mesh, powder, Aldrich Chemical Company). The bottom layer was diluted with distilled water with a ratio of 1:2.2 into a 1000 mL glass Erlenmeyer flask**.** A 25 g of activated carbon was added to

250 ml of the lower portion of solutions, mixed well, and set for 5 min at room temperature. The mixture was filtered through filter paper (Cat No 1001 125, Whatman). The mix and filtration steps were repeated seven times until having transparent (colorless) solutions. Up to 25% of *the volume of the solution* might be *lost* during the preparation.

Color measurements

The color measurements of LaP1 and LaP2 solutions were prepared using the methodology reported by Hammam et al. (2021) with some modifications. The color of the LaP1 and LaP2 solutions was measured using a Minolta Spectrophotometer (CM-508d, Minolta Camera Co., Ltd., Osaka, Japan). Color differences were recorded in the CIE L*a*b* scale in terms of lightness (L*) and color (a* – redness, b* – yellowness) for both solutions. Color measurement was recorded in triplicate, and the average value was recorded. The instrument was calibrated (Y=85.7, x=0.3236, and y=0.3236) with a white calibration plate (CR-210, Konica Minolta, Japan) before being used. Samples were placed in Petri dishes (100 ×15 mm) with an optically clear bottom. A reference sample was done using distilled water in the Petri dishes with clear bottoms. Additionally, the total color difference (ΔE) was calculated using the following formula:

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$$

where $\Delta L^2 + \Delta a^2 + \Delta b^2$ are differences in the L*, a*, and b* values between the reference sample and the test sample, respectively.

Total solids and pH

TS was measured using a forced draft oven (AOAC, 2000, method 990.20; 33.2.44), and pH was determined using the Hanna pH meter (Hannah Edge Blu, Woonsocket, RI 02895).

Statistical Analysis

Statistical analysis was performed to study the difference between treatments in terms of pH, and TS, color. The ANOVA test was done using R software (R×64-3.3.3, R Foundation for Statistical Computing). Mean separation was done using the least significant difference (LSD) test at $P<0.05$.

Results and discussion

Color measurements

Table 1 shows the effect of washing numbers with activated carbon on the color parameters of the LaP1 solutions. The standard L*, a*, and b* were 89.53, -4.97, and 6.07, respectively. On the other hand, the L*, a*, and b* of the down layer of LaP1 were 33.49, 2.17, and -1.85, respectively. In terms of the 1^{st} stage, it appears that the L* of the solution was significantly lower ($P<0.05$) than in the 7^{th} stage. The 6^{th} stage (75.31) was slightly higher than the 7^{th} stage (75.04), but there was no significant difference between those two stages. However, the a* of the 1^{st} wash solution was significantly higher ($P<0.05$) than the 7^{th} wash. It slightly decreased until the 4^{th} wash and then elevated with an increasing number of washings. Furthermore, the b* of the 1^{st} stage was significantly higher ($P<0.05$) than in other stages, which was expected. The a* and b* decreased with

increasing the number of treated solutions with activated carbon; however, L* increased. Januszewicz et al. (2020) found similar results when treating wastewater with activated carbon. Januszewicz's study reported that the color was slightly removed from the water when it was washed multiple times with activated carbon, and this is due to the high surface area of adsorption.

Table 2 illustrates the impact of washing stages with activated carbon on the color of LaP2 solutions. It appears that the L* of the solution of the 1st wash (73.68) was significantly lower ($P<0.05$) than in other washes (81.34 in the 2nd wash vs. 90.21 in the 7th wash). However, the a* of the solution of the 1st wash (-0.41) was significantly higher ($P<0.05$) than the 7th wash (-5.02). It decreased until the 4th stage (-6.34), then slightly increased. Furthermore, the b* significantly dropped ($P<0.05$) from 45.29 in the 1st wash to 6.17 in the 7th wash when the solution was washed with activated carbon. When we compared the 1st stage with the 7th stage of both solutions, the lightness increased, and the redness and yellowness decreased in stage 7. It means that with increasing the number of washes, the dark color slightly decreased, which was expected. A study was done on removing the color of wastewater using activated carbon at different ratios (Hata et al., 2016). The study shows that the efficiency of color removal increased by increasing the dosage of adsorbents.

Tables 3 and 4 present the color removal efficiencies of activated carbons with 7 washes for both LaP1 and LaP2 solutions. It was clear that depending on the wash of activated carbon, the color removal efficiency varied widely among the solutions. Complete color removal was achieved with 7 stages. ΔE of LaP1 solutions was 46.03, 31.13, 16.20, 10.96, 9.27, 14.35, and 14.54 for the 1st, 2nd, 3rd, 4th, 5th, 6th, and 7th stages,

respectively. The ΔE was slightly decreased with increasing the number of washings with activated carbon. The 4th and 5th stages were slightly lower than the 6th and 7th stages. This could relate to a* parameter that was slightly lower ($P>0.05$) in those stages. ΔE of 1st wash was 3 times higher compared to 7th stage. The down layer of solutions was 57.04, which is higher than other stages. On the other hand, ΔE of LaP2 solutions was 42.57, 19.45, 10.43, 8.76, 5.28, 1.40, and 0.68 for the 1st, 2nd, 3rd, 4th, 5th, 6th, and 7th stages of washing, respectively. ΔE was slightly decreased with increasing the number of washings. The LP decolorization performances of activated carbon are shown in Figures 1 and 2. The dark color of both solutions was slightly decreased after multiple times of washing with activated carbon. The color of solutions tended to be brownish (1st and 2nd stages), yellowish (3rd and 4th stages), clear (5th and 6th stages), and transparent (colorless) solutions in the final stages.

pH and TS of solutions

Table 5 shows the effect of activated carbon on the pH and TS of LaP1 solutions. The pH of seven stages was 11.64, 11.61, 11.51, 11.32, 11.05, 10.91, and 10.86, respectively. The pH of solutions slightly decreased ($P<0.05$) with increasing the number of washings. A significant difference was detected between the stages of 4th, 5th, 6th, and 7th and diluted solution before washing with activated carbon (11.72). The pH of the solutions has a critical effect on the decolorization solution using the activated carbon technique. A study on sugar syrup shows that the pH between 4.5 to 6.7 was decolorized easily compared to acidity and alkali conditions (Mudoga et al., 2008). As the solutions were in alkali conditions, this could give activated carbon treatment poor efficiency. This could be related to the hydroxide ions on the solutions, which are competitive ions to

color removal (Obeid et al., 2013). The pH adjustment of solutions before decolorization is recommended to improve the efficiency of activated carbon (Bansal and Goyal, 2005). However, this adjustment of pH could affect the structure of LP.

The TS of the down layer solution was 27.92, which was dilated with distilled water with a ratio of 1:2.2 to obtain 12.61. The TS of the seven stages of washings were 8.43, 7.78, 6.89, 6.11, 5.91, 5.33, and 6.03%, respectively. The TS was slightly decreased with increasing the number of washings, except in stage number 7, which was slightly higher than stage number 6. No significant difference ($P>0.05$) was found in the TS of LaP1 between the 4^{th}, 5^{th}, 6^{th}, and 7^{th} stages. Comparing the TS of diluted solution with the final washing stage, it was significantly higher ($P<0.05$). The loss of TS is related to removing the organic compounds from the solution by the adsorbent technique of activated carbon. It is not entirely clear how the adsorption of organic molecules on activated carbon in an aqueous media. Weber Jr and Morris (1963) theorized that intraparticle diffusion is the critical step in the adsorption of several organic compounds on carbon. In a turbulent environment, several mechanisms are involved in the adsorption of organic compounds on activated carbon.

Table 6 presents the pH and TS of LaP2 solutions before and after being treated with activated carbon. The pH of LaP2 solutions was slightly decreased with the increasing number of washings. The pH of seven stages was 11.48, 11.29, 11.17, 10.89, 10.35, 10.34, and 10.37, respectively. A significant difference ($P<0.05$) was detected between the wash of 5^{th}, 6^{th}, and 7^{th} and the solution after being diluted with distilled water (11.54). This is related to the removal of organic compounds by adsorption techniques, leading to a decreased solution pH (Cendekia et al., 2022). Similar results

were found in Lubis et al. (2020). They purified water using activated carbon from natural sources. They found a decrease in water pH after being treated with activated carbon.

Increased ionization, solubility, and hydrophilicity are typically caused by higher pH (Ma and Uddin, 2013). The adsorption of organic compounds was also discovered to be affected by pH changes by modifying the adsorbents' surface properties and the adsorbate molecules' electronic properties in activated carbon (Yang et al., 2004; Bautista-Toledo et al., 2005). Additionally, it has been demonstrated that the solution pH, which affects the charge density of the activated carbon, significantly impacts the adsorption rate (Zhang et al., 2021). Mohan et al. (2007) studied the commercial activated carbon's ability to be adsorbed from an aqueous phase. The results showed that the adsorption of activated carbon is highly dependent on the pH of the solution; as pH rises from 2.0 to 10.5, adsorption capacity decreases.

The TS of the 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, 6^{th}, and 7^{th} stages of washings was 8.17, 6.78, 6.21, 5.73, 5.76, 5.48, and 4.93%, respectively. TS was slightly decreased with increasing the number of washings. The solution of the 1^{st} wash was significantly higher ($P<0.05$) compared to the 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, 6^{th}, and 7^{th} stages. However, there was no significant difference between the 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, 6^{th}, and 7^{th} stages. It appears that the TS of both solutions was decreased after being treated with activated carbon. Because of its non-polar nature, activated carbon is notably well recognized for its ability to adsorb organic compounds (Bernardo et al., 2006). It is effective in adsorbing different types of chemicals. This led to a decrease in TS of solutions each time treated with activated

carbon. Similar results have found that TS was decreased for beet sugar after being treated with activated carbon (Harris, 1942).

Conclusions

The activated carbon was successfully used to remove the dark color from LaP1 and LaP2 solutions. Both solutions' pH and TS were significantly decreased with increasing the number of washings with activated carbon. When comparing parameters (L*, a*, and b*) of stages, we found that the parameters a* and b* decreased but L* increased. The ΔE was decreased with increasing the number of stages for both solutions. We assume that activated carbon can be used to remove the dark color that results from the phosphorylation process.

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Tables

Table 1. Mean (n=3) variation of color parameters (L*, a*, and b*) of lactose-6-phosphate solutions made with lactose (LaP1) according to the number of washings with activated carbon.

Treatments[1]	Parameters[2]		
	L*	**a***	**b***
Standard	89.53 ±0.31^{a}	-4.97 ±0.03^{c}	6.07 ±0.15^{g}
1st stage	64.98 ±2.01^{e}	2.56 ±0.17^{a}	44.28 ±1.85^{a}
2nd stage	73.96 ±1.93^{d}	-6.31 ±0.17^{e}	32.99 ±0.91^{b}
3rd stage	77.71 ±1.37^{c}	-7.05 ±0.14^{g}	16.95 ±0.46^{c}
4th stage	81.48 ±2.87^{b}	-6.71 ±0.23^{f}	13.30 ±0.58^{d}
5th stage	81.85 ±1.86^{b}	-6.51 ±0.07^{ef}	11.05 ±0.56^{e}
6th stage	75.31 ±1.52^{cd}	-5.71 ±0.09^{d}	7.87 ±0.35^{f}
7th stage	75.04 ±1.56^{cd}	-5.44 ±0.08^{d}	7.16 ±0.16^{fg}
Down layer	33.49 ±0.27^{f}	2.17 ±0.32^{b}	-1.85 ±0.12^{h}

[1]Treatments: Standard= Distilled water into the Petri dish with clear bottom; 1st stage = Lactose solutions after washing 1 time with activated carbon; 2nd stage = Lactose solutions after washing 2 times with activated carbon; 3rd stage = Lactose solution after washing 3 times with activated carbon; 4th stage = Lactose solutions after washing 4 times with activated carbon; 5th stage = Lactose solutions after washing 5 times with activated carbon; 6th stage = Lactose solutions after washing 6 times with activated carbon; 7th stage = Lactose solutions after washing 7 times with activated carbon; down layer= The bottom layer of lactose solution after the phosphorylation process

[2]Parameters: L* = black (0) to white (100); a* = green (-) to red (+); b* = blue (-) to yellow (+)

[a-h] Means in the same row not sharing a common superscript are different at $P<0.05$

Table 2. Mean (n=3) variation of technological parameters of lactose-6-phosphate solutions made with milk permeate (LaP2) according to the number of washings with activated carbon.

Treatments[1]	Parameters[2]		
	L*	a*	b*
Standard	89.53 ± 0.31^{a}	-4.97 ± 0.03^{b}	6.07 ± 0.15^{de}
1st stage	73.68 ± 5.69^{c}	-0.41 ± 3.72^{a}	45.29 ± 3.05^{a}
2nd stage	81.34 ±1.49^{b}	-5.80 ±0.15^{b}	23.70 ±0.87^{b}
3rd stage	87.34 ±1.34^{a}	-6.41 ±0.39^{b}	16.17 ±4.95^{bc}
4th stage	86.61 ±1.20^{a}	-6.34 ±0.23^{b}	14.22 ±2.07^{cd}
5th stage	88.05 ±0.67^{a}	-6.08 ±0.27^{b}	11.02 ±1.41^{cd}
6th stage	89.99 ±0.32^{a}	-5.29 ±0.27^{b}	7.36 ±1.15^{cd}
7th stage	90.21 ±0.38^{a}	-5.02 ±0.08^{b}	6.17 ±0.23^{de}

[1]Treatments: Standard= Distilled water into the Petri dish with clear bottom; 1st stage = Milk permeate solutions after washing 1 time with activated carbon; 2nd stage = Milk permeate solutions after washing 2 times with activated carbon; 3rd stage = Milk permeate solution after washing 3 times with activated carbon; 4th stage = Milk permeate solutions after washing 4 times with activated carbon; 5th stage = Milk permeate solutions after washing 5 times with activated carbon; 6th stage = Milk permeate solutions after washing 6 times with activated carbon; 7th stage = Milk permeate solutions after washing 7 times with activated carbon

[2]Parameters: L* = black (0) to white (100); a* = green (-) to red (+); b* = blue (-) to yellow (+)

[a-e] Means in the same row not sharing a common superscript are different at $P<0.05$

Table 3. Mean (n=3) color determinants and total changes in the color of lactose solutions (LaP1).

Treatments[1]	ΔL^2	Δa^2	Δb^2	ΔE
1st stage	602.70	56.70	1460.26	46.03
2nd stage	242.53	1.79	724.87	31.13
3rd stage	139.71	4.33	118.45	16.20
4th stage	64.86	3.03	52.27	10.96
5th stage	58.98	2.36	24.77	9.27
6th stage	202.21	0.55	3.23	14.35
7th stage	210.15	0.22	1.19	14.54
Down layer	3140.12	50.97	62.72	57.04

[1]Treatments: 1st stage = Lactose solutions after washing 1 time with activated carbon; 2nd stage = Lactose solutions after washing 2 times with activated carbon; 3rd stage = Lactose solution after washing 3 times with activated carbon; 4th stage = Lactose solutions after washing 4 times with activated carbon; 5th stage = Lactose solutions after washing 5 times with activated carbon; 6th stage = Lactose solutions after washing 6 times with activated carbon; 7th stage = Lactose solutions after washing 7 times with activated carbon; down layer= The bottom layer of lactose solution after the phosphorylation process

Equation:

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$$

Table 4. Mean (n=3) color determinants and total changes in the color of milk permeate solutions (LaP2).

Treatments[1]	ΔL^2	Δa^2	Δb^2	ΔE
1st stage	251.33	23.36	1538.21	42.57
2nd stage	67.13	0.69	310.82	19.45
3rd stage	4.81	2.06	102.08	10.43
4th stage	8.55	1.87	66.42	8.76
5th stage	2.21	1.23	24.47	5.28
6th stage	0.21	0.10	1.66	1.40
7th stage	0.45	0.00	0.01	0.68

[1]Treatments:1st stage = Milk permeate solutions after washing 1 time with activated carbon; 2nd stage = Milk permeate solutions after washing 2 times with activated carbon; 3rd stage = Milk permeate solution after washing 3 times with activated carbon; 4th stage = Milk permeate solutions after washing 4 times with activated carbon; 5th stage = Milk permeate solutions after washing 5 times with activated carbon; 6th stage = Milk permeate solutions after washing 6 times with activated carbon; 7th stage = Milk permeate solutions after washing 7 times with activated carbon

Equation:

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$$

Table 5. Mean (n=3) composition of lactose solution (LaP1) after washing with different times of activated carbon.

Treatments[1]	pH	Total solids (%)
Down layer	11.70[a]	27.92[a]
Dilated solution	11.72[a]	12.61[b]
1st stage	11.64[ab]	8.43[c]
2nd stage	11.61[ab]	7.78[c]
3rd stage	11.51[b]	6.89[d]
4th stage	11.32[c]	6.11[de]
5th stage	11.05[d]	5.91[e]
6th stage	10.91[de]	5.33[e]
7th stage	10.86[e]	6.03[de]

[1]Treatments: Down layer= The bottom layer of lactose solution after the phosphorylation process; dilated solution= The bottom layer of lactose solution after the phosphorylation process and dilated with distilled water with a ratio of 1:2.2; 1st stage = Lactose solutions after washing 1 time with activated carbon; 2nd stage = Lactose solutions after washing 2 times with activated carbon; 3rd stage = Lactose solution after washing 3 times with activated carbon; 4th stage = Lactose solutions after washing 4 times with activated carbon; 5th stage = Lactose solutions after washing 5 times with activated carbon; 6th stage = Lactose solutions after washing 6 times with activated carbon; 7th stage = Lactose solutions after washing 7 times with activated carbon

[a-e] Means in the same row not sharing a common superscript are different at $P<0.05$

Table 6. Mean (n=3) composition of milk permeate solution (LaP2) after washing with different times of activated carbon.

Treatments[1]	pH	Total solids (%)
Down layer	11.66[a]	30.12[a]
Dilated solution	11.54[ab]	8.83[b]
1st stage	11.48[ab]	8.17[bc]
2nd stage	11.29[abc]	6.78[bcd]
3rd stage	11.17[bc]	6.21[cd]
4th stage	10.89[c]	5.73[d]
5th stage	10.35[d]	5.76[d]
6th stage	10.34[d]	5.48[d]
7th stage	10.37[d]	4.93[d]

[1]Treatments: Down layer= The bottom layer of milk permeate solution after the phosphorylation process; dilated solution= The bottom layer of milk permeate solution after the phosphorylation process and dilated with distilled water with a ratio of 1:2.2; 1st stage = Milk permeate solutions after washing 1 time with activated carbon; 2nd stage = Milk permeate solutions after washing 2 times with activated carbon; 3rd stage = Milk permeate solution after washing 3 times with activated carbon; 4th stage = Milk permeate solutions after washing 4 times with activated carbon; 5th stage = Milk permeate solutions after washing 5 times with activated carbon; 6th stage = Milk permeate solutions after washing 6 times with activated carbon; 7th stage = Milk permeate solutions after washing 7 times with activated carbon

[a-d] Means in the same row not sharing a common superscript are different at $P<0.05$

Figures

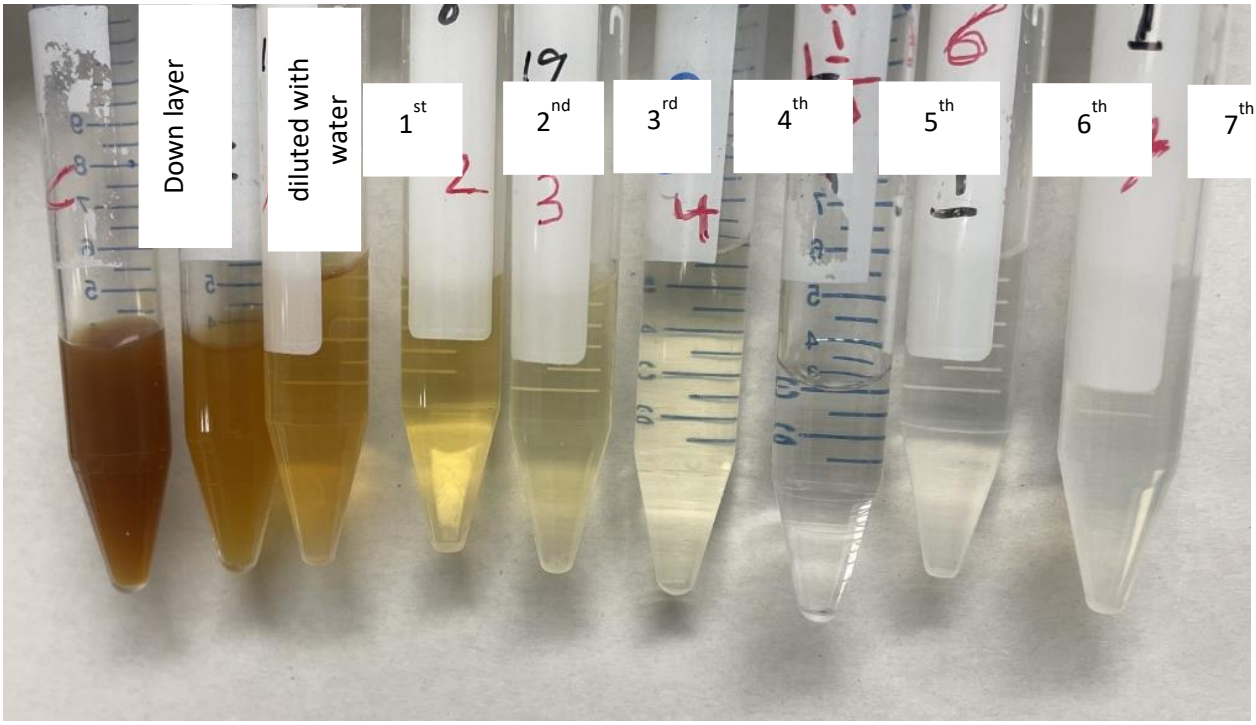

Figure 1. Discoloration of the lactose solution (LaP1) by the application of the activated carbon.

The numbered pictures represent various doses of activated carbon: 1st = Lactose solutions after washing 1 time with activated carbon; 2nd = Lactose solutions after washing 2 times with activated carbon; 3rd = Lactose solution after washing 3 times with activated carbon; 4th = Lactose solutions after washing 4 times with activated carbon; 5th stage = Lactose solutions after washing 5 times with activated carbon; 6th = Lactose solutions after washing 6 times with activated carbon; 7th = Lactose solutions after washing 7 times with activated carbon; down layer= The bottom layer of lactose solution after the phosphorylation process; dilated solution= The bottom layer of lactose after the phosphorylation process and dilated with distilled water with a ratio of 1:2.2

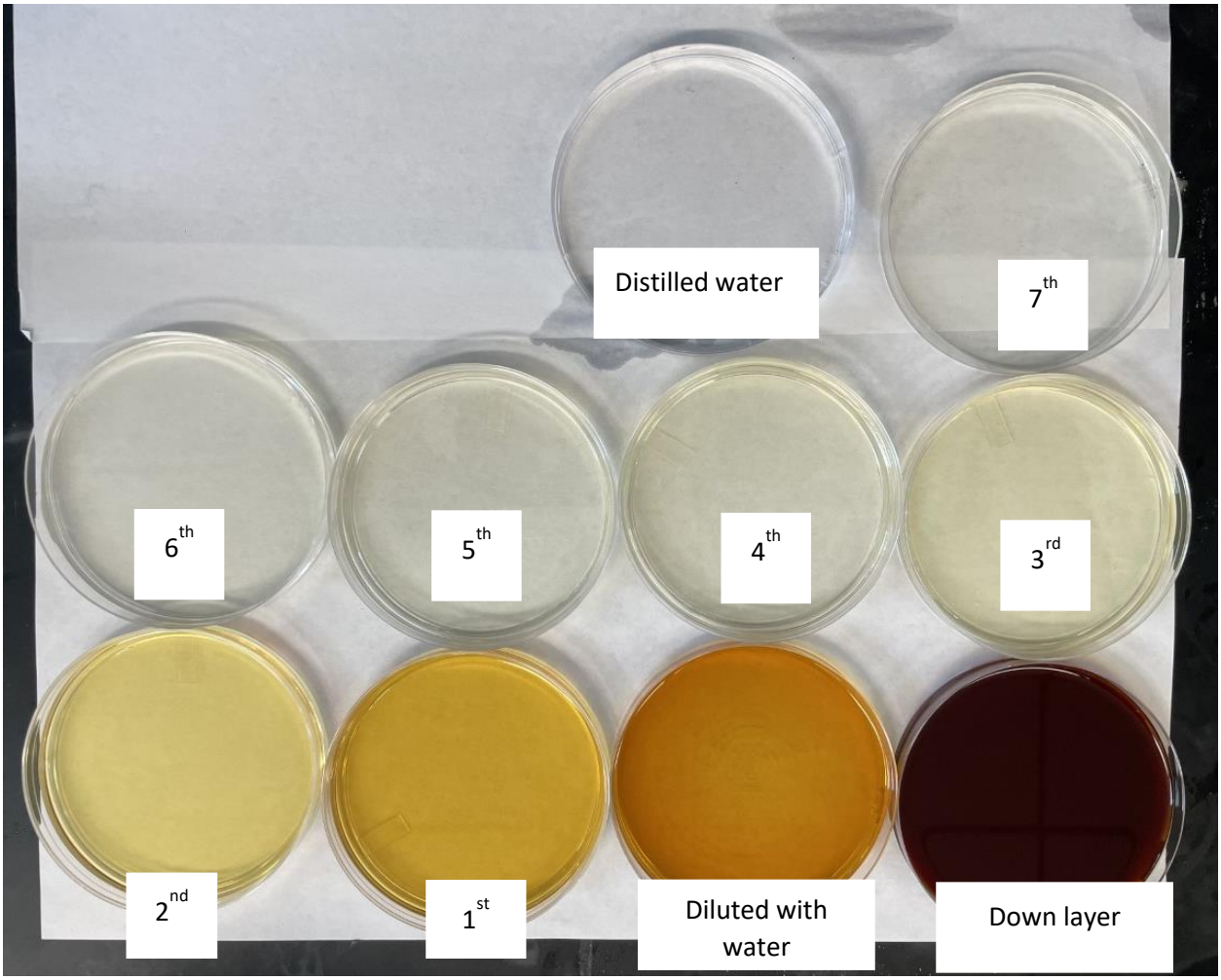

Figure 2. Discoloration of the milk permeate solution (LaP2) by the application of the activated carbon.

The numbered pictures represent various doses of activated carbon:1st= Milk permeate solutions after washing 1 time with activated carbon; 2nd= Milk permeate solutions after washing 2 times with activated carbon; 3rd= Milk permeate solutions after washing 3 times with activated carbon; 4th= Milk permeate solutions after washing 4 times with activated carbon; 5th= Milk permeate solutions after washing 5 times with activated carbon; 6th= Milk permeate solutions after washing 6 times with activated carbon; 7th= Milk permeate solutions after washing 7 times with activated carbon; down layer= The bottom layer of milk permeate solution after the phosphorylation process; dilated solution= The bottom layer of milk permeate solution after the phosphorylation process and dilated with distilled water with a ratio of 1:2.2

CHAPTER V: LACTOSE-6-PHOSPHATE AS AN ALTERNATIVE TO DISODIUM PHOSPHATE IN THE PRODUCTION OF PROCESSED CHEESE FOOD

Abstract

Processed cheese food (PCF) is a dairy product prepared by blending dairy ingredients with non-dairy ingredients and heating the blend with agitation to produce a homogeneous product with an extended shelf-life. Emulsifying salts (ES), such as disodium phosphate (DSP) and trisodium citrate, have a critical effect on the emulsification characteristics of casein by sequestering the calcium from the calcium-paracaseinate phosphate complex in natural cheese (NC). Lactose-6-phosphate (LP) is an organic compound produced from lactose that has the potential to function as ES. The objective of this study was to produce PCF with LP instead of DSP. LP was prepared by mixing 1 mole of α-lactose with 0.5 mole of sodium cyclo-triphosphate. The pH of the solution was adjusted using sodium hydroxide to get a pH of 12. The solution was stirred for 3 d at room temperature and then concentrated to 52% total solids (TS). The ingredients in the PCF formulations were Cheddar cheese, butter, water, milk permeate powder, and LP (at a ratio of 2.0, 2.4, 2.8, 3.2, 4.0, 5.0, and 6.0%) were formulated to contain 17.0% protein, 25.0% fat, 44.0% moisture, and 2.0% salt. PCF made with 2.0% DSP was also produced as a control. The PCF was prepared by mixing all ingredients in a kitchen aid to make a homogeneous paste. A 25 g sample of the mixture was cooked in the rapid visco analyzer for 3 min at 95°C with 1000 rpm for the first 2 min and 160 rpm for the last min. The PCF was then transferred into molds and refrigerated till further analyses. The PCF was analyzed for moisture, pH, end apparent cooked viscosity,

hardness, melted diameter, and melting temperature. The experiment was repeated 3 times using different batches of LP. The moisture of PCF ranged from 42.3 to 44.0%, with a pH of 5.6 to 5.8. The end apparent cooked viscosity increased from 818.0 to 2060.0 cP as the level of LP raised from 2.0 to 6.0%, while it was 660.0 cP in control. The hardness of PCF made with LP elevated from 61.9 to 110.1 g as the level of LP increased; however, it was 85.6 g in control. The melted diameter decreased from 43 mm in control to 29 mm in 6% LP, while the melting temperature of PCF increased from 37.7°C in control to 59.0°C in 6% LP. We conclude that LP can be utilized as a substitute for DSP in PCF manufacture.

Keywords: Processed cheese food; Lactose-6-phosphate; Emulsifying salts; Functional properties

Introduction

Process cheese food (**PCF**) is a dairy product not made directly from milk but is manufactured by blending dairy and non-dairy ingredients at a specific time, temperature, and speed to produce a homogenized product with a long shelf life (Guinee et al., 2004). Diary ingredients include natural cheese (**NC**), whey, butter, anhydrous milk fat, and non-dairy ingredients, such as salt, water, emulsifying salts (**ES**), oil, flavors, and colors. Pasteurized processed cheese (fat ≥ 30%, moisture ≤ 40%, and pH ≥ 5.3), pasteurized processed cheese food (fat ≥ 23%, NC ≥ 51%, moisture ≤ 44%, and pH ≥ 5.0), and pasteurized processed cheese spread (fat ≥ 23%, moisture= 44-60%, and pH ≥ 4.0) are PC categories that differ depending on moisture, fat, pH, and NC contents (Kapoor and Metzger, 2008a).

Emulsifying salts, which consist of monovalent cation and polyvalent anions, are essential in making PCF. Different types of ES can be utilized in the manufacturing of PCF, namely, mono-, di-, trisodium phosphates, dipotassium phosphate, sodium hexametaphosphate, sodium acid pyrophosphate, tetrasodium pyrophosphate, sodium aluminum phosphate, sodium citrate, potassium citrate, calcium citrate, sodium tartrate, and sodium-potassium tartrate. Trisodium citrate and sodium phosphate dibasic (DSP) are commonly used as ES in PCF manufacturing. ES plays an important role in improving the emulsification characteristics of casein by sequestering calcium from the insoluble calcium-paracaseinate-phosphate network in natural cheese or the aggregated casein network in casein containing ingredients. As a result, the sequestered calcium partially disrupts the major molecular forces that cross-link the various casein monomers in the network. The protein is hydrated and dispersed because of this disruption. The partially dispersed monomers of casein have emulsification properties because they have hydrophilic and hydrophobic portions. The hydrophilic portion is linked to the aqueous phase, while the hydrophobic portion is linked to the fat phase, preventing oil separation in the presence of heating and mixing to produce a homogeneous product (Guinee, 2004; Hammam et al., 2022).

Lactose-6-phosphate (LP) is naturally present in milk and milk products with low concentrations (Lifran et al., 2007; Thum et al., 2015). LP was initially identified in bovine milk (Cumar et al., 1965) and was recently identified in caprine milk (Albrecht et al., 2014). It presents in low concentrations, and due to the similar structure to lactose, it is hard to be fractionated and purify. LP is an organic molecule that replaces lactose's hydrogen with monophosphate. A phosphate group is connected to the lactose galactose

portion of most LP molecules. Approximately 90% of LP is coupled to galactose in pharmaceutical-grade lactose, while 10% is bound to glucose (Breg et al., 1988). LP is a chemical molecule generated from lactose that has the potential to behave as ES. It could reduce the amount of sodium and phosphate in PCF. High sodium intake in human nutrition is a risk factor for various disorders, notably causing high blood pressure and heart diseases (Wang and Labarthe, 2011).

Not enough literature on LP is available, and there is a lack of information to understand the composition and structure of this component. Additionally, there was no published data about using LP as ES in PCF. The limitations of studies on LP are as follow: i) Obtaining the right LP combination; ii) Finding pure lactose devoid of LP and other contaminants; iii) Directly analyzing LP and studying the emulsifying properties of LP. Thus, the study hypothesizes using LP as an alternative to ES in PCF manufacture. The main goals of this study were to prepare LP and produce PCF with different concentrations of LP instead of DSP and study the functional properties of the final product.

Materials and methods

Lactose-6-phosphate preparation

LP was prepared using the methodology reported by Inoue et al. (2002) with some modifications. It was prepared by mixing 1 mol of α-D- (+) lactose monohydrate ($C_{12}H_{22}O_{11}.H_2O$: Fisher chemical; L5-500) with 0.5 mol of sodium cyclo-triphosphate (Frontier Scientific; Cat # 343031). The pH of the solution was adjusted using sodium hydroxide to get a pH of 12. The solution was stirred for 3 d at room temperature and

then stored at 4°C for 24 h. The solution was separated into two layers. The top layer was removed using a syringe, and the bottom layer was processed further. The bottom layer was diluted with distilled water with a ratio of 1:2 into a 1000 mL glass Erlenmeyer flask. A 25 g of activated carbon (Darco G60,-100 mesh, powder, Aldrich Chemical Company) was added to 250 ml of the lower portion of LP solution, mixed well, and set for 5 min at room temperature. The mixture was filtered through filter paper (Cat No 1001 125, Whatman). The mix and filtration steps were repeated seven times until having a transparent (colorless) solution. Then, the solution was evaporated at 70°C, 350 mbar, and 100 rpm to obtain LP with 52% total solids (TS). Three different batches of LP were used in this study.

Processed cheese formulations

The PCF formulations (44.0% water, 25.0% fat, 17.0% protein, and 2.0% salt) were prepared using TechWizard software (Metzger, 2010). The ingredients used in making PCF were Cheddar (Great Value, Mild Cheddar Cheese, Bentonville, AR), water, salt (Morton Salt, INC., Chicago, IL), salted butter (Land O Lakes Half Stick salted Butter, INC., Arden Hills, MN), deproteinized whey (Bondgrads' Creameries, Perham, MN), and dibasic sodium phosphate (Fisher Scientific, Fair Lawn, New Jersey) as shown in Table 1.

Mixing ingredients

Cheddar cheese, butter, water, and milk permeate powder were mixed using a Kitchen Aid at room temperature for 30 min to produce a homogenous paste. The mixture from Kitchen Aid was divided into 8 lots of 300 g. The first mix was used as a

control (2% of DSP) (T1). The second (T2), third (T3), fourth (T4), fifth portion (T5), sixth portion (T6), seventh portion (T7), and eighth portion were cooked with 2.0, 2.4, 2.8, 3.2, 4.0, 5.0, and 6.0% of LP solutions. This experiment was repeated three times.

Cooking the processed cheese

The PCF formulations were cooked in the rapid visco analyzer (**RVA**) as described in other studies (Metzger et al., 2002; Kapoor et al., 2004; Kapoor and Metzger, 2005; Hammam et al., 2022). A total of 25 g of the mix with either DSP or LP were transferred to a canister and then tempered at 40°C/15 min in a water bath (SWB-20L-3; Major Science, USA). The canister was cooked in the RVA (Perten RVA 4500, Macquarie Park NSW 2113, Australia). The canister was cooked at 95°C for 3 min at a speed of 1000 rpm for the first two min and 160 rpm during the last min. The end apparent cooked viscosity of PCF was measured at 95°C at the end of the cooking time by calculating the mean of the last five viscosity values, as shown in Figure 1**.** The pH was adjusted between 5.6 to 5.7 using sodium hydroxide 40% w/w (Fisher Scientific, S318-1). PCF was then poured into copper cylinders (20 mm diameter × 30 mm height) and plastic molds (28.3 mm diameter × 25 mm height) sealed with aluminum foil. PCF was refrigerated till further analyses.

Compositional analyses

The final PCF was analyzed for moisture using a forced draft oven (AOAC, 2000, method 990.20; 33.2.44), and pH was determined using the Hanna pH meter **(**Hannah Edge Blu, Woonsocket, RI 02895**).**

Functional analyses

Dynamic Stress Rheometer (DSR)

The dynamic rheological analysis (DSR) determined the initial melt characteristics of PCF and indicated molecular interactions. The melting point is the lowest temperature, where a material shifts from primarily elastic to primarily viscous (Prow and Metzger, 2005). DSR was done as described in a previous study (Hammam et al., 2022) using a rheometer (MSR 92, Anton Paar, Graz, Austria) equipped with a 25-mm parallel plate geometry. The cheese was cut into 2 mm thick slices at room temperature using a wire cutter. A stress sweep test for PCF was performed at a frequency of 1.5 Hz and a range of 1 to 1000 Pa stress at 20°C using the rheometer with parallel plate geometry. The stress sweep test found that the maximum stress limit for the linear viscoelastic region was 500 Pa.

The melting temperature of PCF was determined using the heat rate of 1°C/min. The temperature ranged from 20 to 90°C using a frequency of 1.5 Hz and stress of 500 Pa (linear viscoelastic region). Elastic modulus (G′), viscous modulus (G″), and tangent angle (tan δ). The temperature at which tan δ=1 (G″/ G′) was known as the cheese melt temperature. DSR was done in triplicate.

Schreiber melt test

The melt of PCF was done using the Schreiber melt test. PCF with 28.5 mm diameter was removed from a mold and cut using a wire cutter to obtain 7 mm high. PCF

was transferred to Petri dishes and left at 90 C for 7 min. The diameter of the melted cheese was measured in 4 different spots using a ruler after cooling and reported in mm.

Texture profile analysis (TPA)

The hardness of the PCF was measured using the texture profile analyzer (TPA) as described by (Hammam et al., 2022). A 20 mm high PCF with a 20 mm diameter was cut using a wire cutter and transferred to the texture analyzer (TA.XT-Plus, 6 Patton Drive, South Hamilton, MA). TPA was performed using uniaxial double bite compression (50-mm diameter cylindrical flat probe, 10% compression, and one mm/s crosshead speed). A 20 mm high PCF with a 20 mm diameter was transferred to the TPA. The hardness of PCF was referred to as the top of the first force that results from the first compression. TPA was performed on two samples of each replicate.

Statistical Analysis

Statistical analysis was performed to study the concentration of LP on PCF's functional properties. The ANOVA test was done using R software (R ×64-3.3.3, R Foundation for Statistical Computing). Mean separation was done using the least significant difference (LSD) test at $P< 0.05$.

Results and discussions

Composition of processed cheese

The mean composition of moisture of PCF is illustrated in Table 2. The ANOVA with MS and P-values for moisture of the PCF is exhibited in Table 3. The moisture of

PCF in control, T2, T3, T4, T5, T6, T7, and T8 were 44.0, 42.8, 43.7, 43.3, 43.6, 43.1, 42.3, and 43.1%, respectively. The low moisture content in the final PCF of LP compared to the targeted moisture content (44.0%) could be related to differences in TS of LP that was used in this study, which resulted in decreasing the moisture content in the final PCF. No significant difference ($P > 0.05$) was detected in PCF moisture between control and PCF treated with different concentrations of LP. The moisture of treatments ranged from 42.3 to 44.0%, which is within the expected level for PCF.

The mean composition of PCF's pH is shown in Table 2. The ANOVA with MS and P-values for pH of the PCF is presented in Table 3. The pH of PCF in control, T2, T3, T4, T5, T6, T7, and T8 were 5.6, 5.6, 5.7, 5.7, 5.7, 5.7, 5.7, and 5.8, respectively. The addition of 6.0% LP resulted in the highest pH ($P<0.05$) in the final PCF compared to other treatments. This could be related to the high sodium level in the LP solutions that was noticeable at 6.0% LP. However, no significant difference ($P > 0.05$) was detected in the pH between control and PCF treated with up to 5.0% LP. In this study, PCF's pH ranged from 5.6 to 5.8, which was in the typical range (5.4-5.8) of pH of PCF (Palmer and Sly, 1943; Caric et al., 1985; Marchesseau et al., 1997; Kapoor and Metzger, 2008a). When the pH of the PCF is less than 5.4 or more than 5.8, the stability of PCF emulsion is reduced (Palmer and Sly, 1943). Similar results have been found for some PCF with different concentrations of ES (Gupta et al., 1984). Since the main role of ES is calcium sequestration and pH adjustment, which increase with the increase in the amount of ES, these interactions improve the final product's homogenization by hydrating caseins and increasing the interaction between water and fat phases (Kapoor and Metzger, 2008a). The slight differences in pH could affect the viscosity of PCF (Caric et al., 1985).

Functional Properties

End apparent cooked viscosity

The mean values of cooked viscosity (cP) of PCF measured using the RVA are exemplified in Table 4. The ANOVA with MS and P-values for cooked viscosity of the PCF is illustrated in Table 5. The viscosity of PCF in control, T2, T3, T4, T5, T6, T7, and T8 were 660.5, 818.9, 1065.3, 1205.3, 1175.2, 1691.9, and 2060.5 cP, respectively. There was a significant difference ($P<0.05$) in the end apparent cooked viscosity of PCF made with different levels of LP. This could be due to the differences in the amount of sodium coming from LP that affect the pH. The variations in the pH of the final PCF might impact the end apparent cooked viscosity of PCF. When the pH of PCF elevated, the net negative charges of casein increased, and this, in turn, led to increases in the calcium-mediated cross-linking of casein molecules in the PCF gel network. When PCF is heated, the calcium-induced cross-linking of casein molecules restricts the movement of the casein chains, reducing flowability and increasing viscosity (Kapoor and Metzger, 2008; Marchesseau et al., 1997). Similar results were reported by Kapoor (2007), who noticed increasing the viscosity of PCF with increasing pH to 5.8.

Hardness

The mean values of hardness (g) of PCF determined using the TPA are revealed in Table 4. The ANOVA with MS and P-values for the hardness of the PCF is displayed in Table 5. The hardness of PCF in control, T2, T3, T4, T5, T6, T7, and T8 was 85.6, 64.1, 66.3, 61.9, 72.4, 67.4, 77.8, and 110.1 g, respectively. Significant differences ($P<0.05$) were detected in the hardness of PCF made with different levels of LP. No

significant difference ($P > 0.05$) was detected between PCF made with 2% DSP (control) and PCF treated with LP up to 5.0%. The highest hardness was found in PCF made with 6% LP, which could relate to the pH of the final PCF. PCF's pH significantly impacts its rheology, texture, and microstructure characteristics (Mulsow et al., 2007). As the pH of PCF increases, the net negative charges on caseins and the electrostatic repulsion in the casein matrix elevate. Higher repulsion at high pH should result in a more open and loose PCF network with improved water binding capacity and emulsifying ability during PCF manufacturing (Guinee et al., 2004). When increasing the pH of PCF, the hydrophobic interactions between individual casein molecules should decrease due to the increase in electrostatic repulsion (Horne, 1998; Lucey et al., 2003). This results in stronger hydrogen bonds and more calcium-mediated cross-links between casein molecules in PCF, which improves the strength of the PCF gel network and hence the hardness of the final PCF (Bulut-Solak and Akin, 2019). Another study found that the final pH of PCF affected its firmness (Templeton and Sommer, 1932; Marchesseau et al., 1997). They discovered that as the PCF's pH increased from 5.0 to 6.2, its firmness improved up to around pH 5.8 (where it had the most firmness), but the firmness started to drop as the pH increased further from 5.8 to 6.2.

Schreiber melt test

The mean values of the Schreiber melt test of PCF made with LP solutions are presented in Table 6. The ANOVA with MS and P-values for the Schreiber melt test (mm) of the PCF is demonstrated in Table 7. The Schreiber melt test of PCF made with 2% DSP (control), T2, T3, T4, T5, T6, T7, and T8 were 43.2, 34.2, 30.5, 32.5, 31.1, 30.4, 32.6, and 29.3 mm, respectively. The Schreiber melt test of PCF made from DSP was

significantly different ($P < 0.05$) compared to PCF made with LP. The high melting diameter was noticed in the control, while the lowest melted diameter was found in PCF made with 6% LP, and this finding was correlated with the pH data. When increasing the pH of PCF resulted from the high concentration of LP, the hydrophobic interactions between individual casein molecules reduced due to the increase in electrostatic repulsion (Horne, 1998; Lucey et al., 2003). This results in stronger hydrogen bonds and more calcium-mediated cross-links between casein molecules in PCF, which improves the PCF gel network's strength and decreases the final PCF's melting area (Bulut-Solak and Akin, 2019).

Melting temperature

The mean values of melting temperature (°C) of PCF made with 2% DSP and LP at different levels are displayed in Table 6. The ANOVA with MS and P-values for melt temperature (°C) of the PCF is shown in Table 7. The melting temperature of PCF in control, T2, T3, T4, T5, T6, T7, and T8 was 37.7, 43.00, 51.10, 53.00, 49.1, 55.4, 57.90, and 58.9°C, respectively. There was a significantly different ($P<0.05$) between treatments in the melting point of PCF. The low melting temperature was found in control, while the high melting temperature was revealed in PCF made with 6% LP, and this finding was correlated with the pH data. The higher pH in the final PCF, the greater the melt temperature. The low melting point is related to the higher amount of insoluble calcium (Shirashoji et al., 2016) and low pH due to the poor emulsification of fat and water at low pH. Also, this may be related to the level and buffering capacity of ES used on PCF, which plays a critical role in the melting points of PCF (Marchesseau et al., 1997).

Conclusions

LP solution was prepared and used successfully as an alternative to DSP in making PCF. When comparing PCF at the same pH and ES content, we assume that the higher the degree of casein dispersion caused by using a certain type of ES during cooking, the firmer the product after cooling. This study concluded that as LP concentration elevated, the pH increased, which had a significant impact on PCF properties. This study determined that LP could replace DSP to produce PCF with less melt than DSP. The concentration of LP can be adjusted based on the required functional characteristics of PCF. The level of LP has a significant impact on the physical and chemical properties of PCF. The study found that the higher the LP, the more significant viscosity, hardness, melt temperature, and lower melting area.

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Tables

Table 1. Mean (n=3) of processed cheese food (PCF) formulations (g $100g^{-1}$).

Treatments[1]	Ingredients						
	Chaddar cheese	**Water**	**Salted Butter**	**Deproteinized whey**	**Salt**	**Sodium phosphate dibasic**	**Lactose-6-phosphate**
Control	66.95	18.10	3.45	7.45	2	2	-
T2	66.95	18.12	3.45	7.45	2	-	2
T3	67.01	18.05	3.43	7.10	2	-	2.4
T4	67.07	17.97	3.41	6.74	2	-	2.8
T5	67.13	17.89	3.38	6.38	2	-	3.2
T6	67.24	17.74	3.33	5.67	2	-	4
T7	67.39	17.57	3.28	4.77	2	-	5
T8	67.46	16.93	3.25	4.34	2	-	6

[1] Treatments: Control= Processed cheese made with sodium phosphate dibasic; T2= Processed cheese made with 2% lactose-6-phosphate solutions; T3= Processed cheese made with 2.4% lactose-6-phosphate solutions; T4= Processed cheese made with 2.8% lactose-6-phosphate solutions; T5= Processed cheese made with 3.2% lactose-6-phosphate solutions; T6= Processed cheese made with 4% lactose-6-phosphate solutions; T7= Processed cheese made with 5% lactose-6-phosphate solutions; and T8= Processed cheese made with 6% lactose-6-phosphate solutions

Table 2. Mean (n=3) composition of processed cheese (PCF) made with different levels of lactose-6-phosphate (LP).

Composition	Treatment[1]							
	Control	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**
Moisture (%)	44.0[a]	42.8 [a]	43.7[a]	43.3[a]	43.6[a]	43.1[a]	42.3[a]	43.1[a]
pH	5.6[a]	5.6[a]	5.7[a]	5.7[a]	5.7[a]	5.7[a]	5.7[a]	5.8[b]

[1] Treatments: Control= Processed cheese made with sodium phosphate dibasic; T2= Processed cheese made with 2% lactose-6-phosphate solutions; T3= Processed cheese made with 2.4% lactose-6-phosphate solutions; T4= Processed cheese made with 2.8% lactose-6-phosphate solutions; T5= Processed cheese made with 3.2% lactose-6-phosphate solutions; T6= Processed cheese made with 4% lactose-6-phosphate solutions; T7= Processed cheese made with 5% lactose-6-phosphate solutions; and T8= Processed cheese made with 6% lactose-6-phosphate solutions

[a-b] Means in the same row not sharing a common superscript are different at $P < 0.05$

Table 3. Mean squares and P-values of pH and moisture of processed cheese (PCF) with either Lactose-6-phosphate (LP) or sodium phosphate dibasic (DSP).

Factor	**df**	**pH**	**Moisture**
Replication	2	0.004 (0.29)	11.32 (<0.05)
Treatment	7	0.009 (<0.05)	0.92 (0.74)
Error	14	0.003	1.53

Table 4. Mean (n=3) viscosity and hardness (g) of processed cheese (PCF) with either Lactose-6-phosphate (LP) or sodium phosphate dibasic (DSP).

Parameter	**Treatments[1]**							
	Control	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**
Viscosity (cP)	660.5^{e}	818.9^{de}	1065.3^{cd}	1205.3^{c}	1152.9^{c}	1175.2^{c}	1691.9^{b}	2060.5^{a}
Hardness (g)	85.6^{ab}	64.1^{b}	66.3^{b}	61.9^{b}	72.4^{ab}	67.4 ^{b}	77.8^{ab}	110.1^{a}

[1] Treatments: Control= Processed cheese made with sodium phosphate dibasic; T2= Processed cheese made with 2% lactose-6-phosphate solutions; T3= Processed cheese made with 2.4% lactose-6-phosphate solutions; T4= Processed cheese made with 2.8% lactose-6-phosphate solutions; T5= Processed cheese made with 3.2% lactose-6-phosphate solutions; T6= Processed cheese made with 4% lactose-6-phosphate solutions; T7= Processed cheese made with 5% lactose-6-phosphate solutions; and T8= Processed cheese made with 6% lactose-6-phosphate solutions

[a-d] Means in the same row not sharing a common superscript are different at $P < 0.05$

Table 5. Mean squares and P-values of end apparent cooked viscosity and hardness of process cheese food (PCF) with either lactose-6-phosphate (LP) or disodium phosphate (DSP).

Factor	**df**	**End apparent cooked viscosity**	**Hardness**
Replication	2	220328 (<0.05)	578.14 (0.32)
Treatment	7	614181 (<0.05)	761.27 (0.21)
Error	14	27724	467.38

Table 6. Mean (n=3) Schreiber melt test (mm) and melting temperature (°C) of processed cheese (PCF) with different levels of lactose-6-phosphate (LP).

Parameter	**Treatments[1]**							
	Control	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**
Schreiber melt test (mm)	43.2^{a}	34.2 ^{b}	30.5^{cd}	32.5^{bc}	31.1^{cb}	30.4^{cb}	32.6^{bcd}	29.3^{d}
Melting temperature (°C)	37.7^{e}	43.0^{de}	51.1^{bc}	53.0^{abc}	49.1^{cd}	55.4^{abc}	57.9^{ab}	58.9^{a}

[1] Treatments: Control= Processed cheese made with sodium phosphate dibasic; T2= Processed cheese made with 2% lactose-6-phosphate solutions; T3= Processed cheese made with 2.4% lactose-6-phosphate solutions; T4= Processed cheese made with 2.8% lactose-6-phosphate solutions; T5= Processed cheese made with 3.2% lactose-6-phosphate solutions; T6= Processed cheese made with 4% lactose-6-phosphate solutions; T7= Processed cheese made with 5% lactose-6-phosphate solutions; and T8= Processed cheese made with 6% lactose-6-phosphate solutions

[a-d] Means in the same row not sharing a common superscript are different at $P < 0.05$.

Table 7. Mean squares and P-values of Schreiber melt test (mm) and melting temperature (°C) of process cheese food (PCF) with either lactose-6-phosphate (LP) or disodium phosphate (DSP).

Factor	**df**	**Diameter**	**DSR**
Replication	2	0.849 (0.76)	6.56 (0.67)
Treatment	7	59.19 (<0.05)	161.81 (<0.05)
Error	14	3.001	16.43

Figures

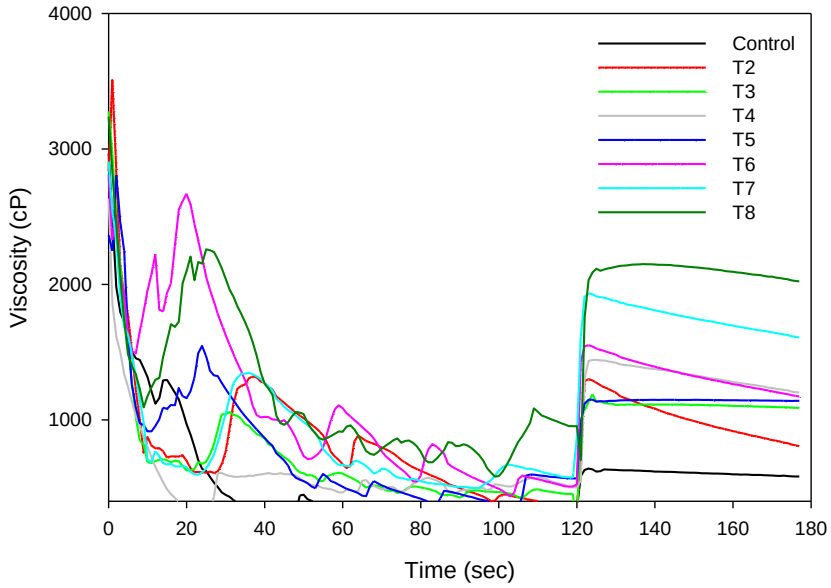

Figure 1. Measuring the apparent cooked viscosity of process cheese food using the rapid visco analyzer (RVA).

[1] Treatments: Control= Processed cheese made with sodium phosphate dibasic; T2= Processed cheese made with 2% lactose-6-phosphate solutions; T3= Processed cheese made with 2.4% lactose-6-phosphate solutions; T4= Processed cheese made with 2.8% lactose-6-phosphate solutions; T5= Processed cheese made with 3.2% lactose-6-phosphate solutions; T6= Processed cheese made with 4% lactose-6-phosphate solutions; T7= Processed cheese made with 5% lactose-6-phosphate solutions; and T8= Processed cheese made with 6% lactose-6-phosphate solutions

CHAPTER VI: MANUFACTURE OF LACTOSE-6-PHOSPHATE FROM MILK PERMEATE: A SUBSTITUTE FOR DISODIUM PHOSPHATE IN MAKING PROCESSED CHEESE FOOD

Abstract

Processed cheese food (PCF) is a dairy product made by mixing dairy with non-dairy ingredients and then heating the mixture with agitation to create a homogenous product with a long shelf life. Lactose-6-phosphate (LP) is a lactose-derived chemical molecule with the potential to function as an emulsifying salt. This work aimed to develop a method to produce LP using milk permeate powder (MPP) and the produced LP solution in PCF manufacturing. LP was prepared by dissolving MPP and disodium phosphate (DSP) in distilled water at 28.32 and 1.41%, respectively. The pH of the solution was adjusted using sodium hydroxide to a pH of 12. The solution was stirred for 3 d at room temperature and then stored at 4°C for 24 h. The solution was separated into two layers, and the bottom layer was utilized for further analyses. It was diluted with distilled water with a ratio of 1:2.2. Activated carbon was used to remove the brown color from the solution. Activated carbon was mixed and set for 5 min at room temperature, and then it was filtered. Those washing and filtration steps were repeated 7 times until having a transparent (colorless) solution. The solution was then evaporated to achieve 70% total solids in the final solution. The ingredients in the PCF formulations were Cheddar cheese, butter, water, MPP, and LP (8.0%). Those ingredients were formulated to contain 17.0% protein, 25.0% fat, 43.0% moisture, and 2.0% salt. PCF with 2.5% DSP was also produced as a control. The experiment was repeated 5 times using 5 different batches of LP. The moisture of PCF ranged from 42.61 and 43.09%. The pH was 5.81 for

PCF made with LP; however, it was 5.74 in control. The cooked viscosity of LP was 2032.0 cP, while it was 1378.0 cP in control. The hardness of PCF made with LP was 154.5 g and 91.6 g in control. The melted diameter decreased from 41.0 mm in control to 34.0 mm in LP, while the melting temperature of PCF increased from 43.2°C in control to 46.5°C in LP. We conclude that LP can be produced using MPP and utilized as a substitute for DSP in PCF manufacture.

Keywords: Processed cheese food; Lactose-6-phosphate; Emulsifying salts; Milk permeate; Functional properties

Introduction

Processed cheese food (PCF) is a stable oil-in-water emulsion. It is made by blending natural cheese (NC) in the presence of emulsifying salts (ES) and other dairy and non-dairy ingredients, then heating and mixing to create a homogenous product with a long shelf life. Pasteurized processed cheese (fat ≥ 30%, moisture ≤ 40%, and pH ≥ 5.3), pasteurized processed cheese food (fat ≥ 23%, NC ≥ 51%, moisture ≤ 44%, and pH ≥ 5.0), and pasteurized processed cheese spread (fat ≥ 23%, moisture= 44-60%, and pH ≥ 4.0) are PC categories that differ depending on moisture, fat, pH, and NC contents (Kapoor and Metzger, 2008a).

ES, which is composed of a monovalent cation and polyvalent anions, is required for the production of PCF. Mono-, di-, and trisodium phosphates, dipotassium phosphate, sodium hexametaphosphate, sodium acid pyrophosphate, tetrasodium pyrophosphate, sodium aluminum phosphate, sodium citrate, potassium citrate, calcium citrate, sodium tartrate, and sodium-potassium tartrate are examples of ES that can be utilized in the

manufacture of PCF. Trisodium citrate and sodium phosphate dibasic (DSP) are commonly used as ES in PCF manufacturing. ES plays an essential role in improving the emulsification characteristics of casein by sequestering calcium from the insoluble calcium-paracaseinate-phosphate network in NC or the aggregated casein network in casein-containing ingredients. As a result, the sequestered calcium disrupts the major molecular forces that cross-link the network's casein monomers. Because of this disruption, the protein is hydrated and dispersed. The partially dispersed monomers of casein have emulsification properties because they have hydrophilic and hydrophobic portions. The hydrophilic portion is linked to the aqueous phase, while the hydrophobic portion is linked to the fat phase, preventing oil separation and producing a homogeneous product with heating and mixing (Guinee, 2004; Hammam et al., 2022).

Milk permeate (MP) consists of water and milk solutes of low molecular weight, including minerals, vitamins, whey proteins, and lactose (Fitzpatrick and O'Keeffe, 2001). Milk permeate powder (MPP) contains lactose, protein, and ash at a concentration of 84.6, 2.98, and 7.48%, respectively (Tsermoula et al., 2021). The main compound in milk permeate is lactose. Massive quantities of MP generated by the dairy industry have shifted from being a significant waste concern to being employed in various ways to provide value to the sector. In the dairy industry, considerable quantities of MP are produced by the ultrafiltration (**UF**) process used to concentrate milk's fat and protein content (Cuartas-Uribe et al., 2009). MP is employed in animal feed, land fertilizer, and lactose powder manufacturing (Parashar et al., 2016). Since the Codex Alimentarius Commission permitted the standardization of milk's protein content in 1999, permeate

has been used extensively as a mixing agent in the protein standardization of milk (Tsermoula et al., 2021).

Sugar phosphorylation has many applications that can be used to develop dairy and food products. Increasing the hydrophilicity of starch through phosphorylation is a common chemical modification method that produces products useful for the food, paper, glue, textile, and pharmaceutical industries (Solarek, 1986; Chiu and Solarek, 2009). Previous studies' findings demonstrated that the phosphorylation approach had been successfully used to enhance the attributes of mung bean, rice, corn, wheat, sago, and other starches (Lim and Seib, 1993; Muhammad et al., 2000; Lin et al., 2009; Nathania et al., 2017).

Lactose-6-phosphate (LP) exists naturally in low concentrations in milk and milk products (Lifran et al., 2007; Thum et al., 2015). LP was first discovered in bovine milk (Cumar et al., 1965) and was recently discovered in caprine milk (Albrecht et al., 2014). It is found in low concentrations, and due to its similar structure to lactose, it is challenging to fractionate and purify. LP is an organic molecule that replaces the hydrogen in lactose with monophosphate. Most LP molecules have a phosphate group attached to the lactose galactose portion. In pharmaceutical-grade lactose, approximately 90% of the LP is coupled to galactose, while 10% is bound to glucose (Breg et al., 1988). LP is a lactose-derived chemical molecule with the potential to behave as ES. It can potentially reduce the amount of sodium and phosphate in PCF. High sodium consumption in human nutrition is a risk factor for various disorders, most notably high blood pressure and heart disease (Wang and Labarthe, 2011).

Not enough literature on LP is available, and there is a lack of information to understand the composition and structure of this component. Additionally, there was no published data about using LP as ES in PCF. The limitations of studies on LP are as follow: i) Obtaining the right LP combination; ii) Finding pure lactose devoid of LP and other contaminants; iii) Directly analyzing LP and studying the emulsifying properties of LP. Thus, this study hypothesizes using LP as an alternative to ES in PCF manufacture. The main goals of this study were to prepare LP and produce PCF with 8% of LP instead of DSP and to study the functional properties of the final product.

Materials and methods

Lactose-6-phosphate preparation

With some modifications, LP was prepared using the methodology reported by Inoue et al. (2002). LP was prepared by mixing MPP obtained from Idaho Milk Products (Jerome, ID, USA) and sodium phosphate dibasic (DSP) (Fisher Scientific, Fair Lawn, New Jersey) in distilled water at a concentration of 28.32 and 1.41%, respectively. The pH of the solution was adjusted using sodium hydroxide to get a pH of 12. The solution was stirred for 3 d at room temperature and then stored at 4°C for 24 h. The solution was separated into two layers. The top layer was removed using a syringe, and the bottom layer was processed further. The bottom layer was diluted with distilled water with a ratio of 1:2.2 into a 1000 mL glass Erlenmeyer flask. A 25 g of activated carbon (Darco G60,-100 mesh, powder, Aldrich Chemical Company) was added to 250 ml of LP solution, mixed well, and set for 5 min at room temperature. The mixture was filtered through filter paper (Cat No. 1001 125, Whatman). The mixed and

filtration steps were repeated seven times until having a transparent (colorless) solution. Then, the solution was evaporated using a rotary evaporator (Heidolph Rotary Evaporator with WB eco bath; Schwabach, Germany) at 70°C, 350 mbar, and 100 rpm to obtain LP with 70% total solids (TS). Five different batches of LP solutions were used in this study.

Processed cheese food formulations

The PCF formulations (44.0% water, 25.0% fat, 17.0% protein, and 2.0% salt) were prepared using TechWizard software (Metzger, 2010). The ingredients used in making PCF were Cheddar (Great Value, medium Cheddar Cheese, Bentonville, AR), water, salt (Morton Salt, INC., Chicago, IL), salted butter (Land O Lakes Half Stick salted Butter, INC., Arden Hills, MN), deproteinized whey (Bondgrads' Creameries, Perham, MN), and dibasic sodium phosphate (Fisher Scientific, Fair Lawn, New Jersey) as shown in Table 1.

Mixing ingredients

Cheddar cheese, butter, water, and MPP were mixed using a Kitchen Aid at room temperature for 30 min to produce a homogenous paste. Two different formulations were prepared. The first formulation was produced using 2.5% of DSP as a control. The second formulation was manufactured using 8% LP solutions (70% TS). This experiment was repeated five times with different LP solutions.

Cooking the processed cheese food

As described in other studies, the PCF formulations were cooked in the rapid visco analyzer (**RVA**; Perten RVA 4500, Macquarie Park NSW 2113, Australia)

(Metzger et al., 2002; Kapoor et al., 2004; Kapoor and Metzger, 2005; Hammam et al., 2022). A total of 25 g of mixed cheese with either DSP or LP were transferred to a canister and then tempered at 40°C /15 min in a water bath (SWB-20L-3; Major Science, USA). The canister was cooked in RVA at 95°C for 3 min at a speed of 1000 rpm for the first two min and 160 rpm during the last min. The end apparent cooked viscosity of PCF was measured at 95°C at the end of the cooking time by calculating the mean of the last five viscosity values, as shown in Figure 1**.**

The pH was adjusted between 5.7 to 5.8 using either sodium hydroxide 40% w/w (Fisher Scientific, S318-1) or lactic acid (concentration 85%, Fisher Scientific, Pittsburgh, PA). PCF was pulled into copper cylinders (20 mm diameter × 30 mm height) and plastic molds (28.3 mm diameter × 25 mm height) and sealed with aluminum foil. PCF was refrigerated till further analyses.

Compositional analyses

The final PCF was analyzed for moisture using a forced draft oven (AOAC, 2000, method 990.20; 33.2.44), and pH was determined using the Hanna pH meter **(**Hannah Edge Blu, Woonsocket, RI 02895**).**

Functional analyses

Dynamic Stress Rheometer (DSR)

The dynamic rheological analysis (**DSR**) determined the initial melt characteristics of PCF and indicated molecular interactions. The melting point is the lowest temperature, where a material shifts from primarily elastic to primarily viscous

(Prow and Metzger, 2005). DSR was done as described in a previous study (Hammam et al., 2022) using a rheometer (MSR 92, Anton Paar, Graz, Austria) equipped with a 25-mm parallel plate geometry. The cheese was cut into 2 mm thick slices at room temperature using a wire cutter. A stress sweep test for PCF was performed at a frequency of 1.5 Hz and a range of 1 to 1000 Pa stress at 20°C using the rheometer with parallel plate geometry. The stress sweep test found that the maximum stress limit for the linear viscoelastic region was 500 Pa.

The temperature ranged from 20 to 90°C at a rate of 1°C/min using a frequency of 1.5 Hz and stress of 500 Pa (linear viscoelastic region). Elastic modulus (G′), viscous modulus (G″), and tangent angle (tan δ) were recorded during the test. The temperature at which tan δ=1 (G″/ G′) was known as the cheese melt temperature. DSR was done two times for each replicate.

Schreiber melt test

The melt of PCF was done by using the Schreiber melt test. PCF with 28.5 mm diameter was removed from the mold and cut using a wire cutter to obtain 7 mm high. PCF was transferred to Petri dishes and left at 90 C for 7 min. The diameter of the melted cheese was measured in 4 different spots using a ruler after cooling and reported in mm.

Texture profile analysis (TPA)

The hardness of the PCF was measured using the texture profile analyzer (**TPA**) as described by Hammam et al. (2022). A 20 mm high PCF with a 20 mm diameter was cut using a wire cutter and transferred to the texture analyzer (TA.XT-Plus, 6 Patton

Drive, South Hamilton, MA). TPA was performed using uniaxial double bite compression (50-mm diameter cylindrical flat probe, 10% compression, and one mm/s crosshead speed). A 20 mm high PCF with a 20 mm diameter was transferred to the TPA. The hardness of PCF was the maximum force of the first compression. TPA was performed on two samples of each replicate.

Statistical Analysis

Statistical analysis was performed to study the effect of LP on the functional properties of PCF. One way ANOVA test was done using R software (R ×64-3.3.3, R Foundation for Statistical Computing). Mean separation was done using the least significant difference (LSD) test at $P< 0.05$.

Results and discussion

Composition of processed cheese food

The mean composition of PCF's moisture is illustrated in Table 2. The ANOVA with MS and P-values for moisture of the PCF is shown in Table 3. The moisture of PCF in control and LP were 42.6 and 43.1, respectively. No significant difference ($P> 0.05$) was detected in PCF moisture between control and PCF treated with LP solutions. The moisture of treatments ranged from 42.6 to 43.1%, which is within the expected level for PCF. The low moisture content in the final PCF compared to the targeted moisture content (44.0%) could be related to the evaporation of some water during cooking the PCF in RVA, which resulted in lower moisture content in the final PCF compared to the target.

The mean composition of PCF's pH is demonstrated in Table 2. The ANOVA with MS and P-values for pH of the PCF is presented in Table 3. The pH of PCF made with DSP and LP were 5.7 and 5.8, respectively. A significant difference ($P < 0.05$) was detected in the pH between control and PCF treated with LP. This could be related to the high sodium level in the LP solutions. In this study, The pH of PCF ranged from 5.7 to 5.8, which was in the typical range (5.4-5.8) of PCF's pH (Palmer and Sly, 1943; Caric et al., 1985; Marchesseau et al., 1997; Kapoor and Metzger, 2008a). When the pH of the PCF is less than 5.4 or more than 5.8, the stability of PCF emulsion is reduced (Palmer and Sly, 1943). Similar results have been found for some PCF with different concentrations of ES (Gupta et al., 1984). The slight differences in pH could affect the functional properties of PCF (Caric et al., 1985).

Functional Properties

End apparent cooked viscosity

The mean values of cooked viscosity (cP) of PCF measured using the RVA are exemplified in Table 4. The ANOVA with MS and P-values for cooked viscosity of the PCF is illustrated in Table 5. The viscosity of PCF in control and LP were 1378.0 and 2032.0 cP, respectively. A significant difference ($P< 0.05$) was detected in the end apparent cooked viscosity of control and LP.

The differences in end apparent cooked viscosity could be due to the variations in the amount of sodium coming from LP that affect the pH. The differences in the pH of the final PCF might impact the end apparent cooked viscosity of PCF. When the pH of PCF elevated, the net negative charges of casein increased, and this, in turn, led to

increases in the calcium-mediated cross-linking of casein molecules in the PCF gel network. When PCF is heated, the calcium-induced cross-linking of casein molecules restricts the movement of the casein chains, reducing flowability and increasing viscosity (Kapoor and Metzger, 2008; Marchesseau et al., 1997). Similar results were reported by Kapoor (2007), who noticed increasing the viscosity of PCFF from 1844 to 2077 when the pH elevated from 5.48 to 5.78.

Hardness

The mean values of hardness (g) of PCF determined using the TPA are revealed in Table 4. The ANOVA with MS and P-values for the hardness of the PCF is displayed in Table 5. The hardness of PCF in control and LP were 91.61 and 154.55 g, respectively. No significant difference ($P > 0.05$) was detected between PCF made with 2.5% DSP (control) and PCF treated with LP (8.0%). The high hardness was found in PCF made with 8.0% LP, which could relate to the pH of the final PCF. PCF's pH significantly impacts its rheology, texture, and microstructure characteristics (Mulsow et al., 2007). As the pH of PCF increases, the net negative charges on caseins and the electrostatic repulsion in the casein matrix elevate. When increasing the pH of PCF, the hydrophobic interactions between individual casein molecules should decrease due to the increase in electrostatic repulsion (Horne, 1998; Lucey et al., 2003). This results in stronger hydrogen bonds and more calcium-mediated cross-links between casein molecules in PCF, which improves the strength of the PCF gel network and hence the hardness of the final PCF (Bulut-Solak and Akin, 2019). Another study found that the final pH of PCF affected its firmness (Templeton and Sommer, 1932; Marchesseau et al., 1997). They discovered that as the PCF's pH increased from 5.0 to 6.2, its firmness improved to

around pH 5.8 (where it had the most firmness), but the firmness started to drop as the pH increased further from 5.8 to 6.2. Higher repulsion at high pH should result in a more open and loose PCF network with less hardness (Sheehan and Guinee, 2004).

Schreiber melt test

The mean values of the Schreiber melt test of PCF made with LP solutions are presented in Table 6. The ANOVA with MS and P-values for the Schreiber melt test (mm) of the PCF is demonstrated in Table 7. The Schreiber melt test of PCF made with 2.5% DSP (control), and PCF treated with LP solutions were 41.18 and 34.08 mm, respectively. The Schreiber melt test of PCF made from DSP was significantly higher ($P<0.05$) than PCF made with LP. This finding was correlated with the pH data. Increasing the pH of PCF made with LP resulted in stronger hydrogen bonds and more calcium-mediated cross-links between casein molecules in PCF, which improves the PCF gel network's strength and decreases the final PCF's melting area (Horne, 1998; Lucey et al., 2003; Bulut-Solak and Akin, 2019).

Melting temperature

The mean values of melting temperature (°C) of PCF made with 2.5% DSP and LP are shown in Table 6. The ANOVA with MS and P-values for melt temperature (°C) of the PCF are displayed in Table 7. The melting temperature of PCF made using 2.5% DSP (control) and LP were 43.23 and 46.58°C, respectively. No significant difference ($P>0.05$) was detected between PCF made with DSP and LP. The PCF made with LP had a slightly higher melting point than the control. The low melting point is related to the higher amount of insoluble calcium (Shirashoji et al., 2016) and low pH due to the poor

emulsification of fat and water. The melting temperature results correlate with the Schreiber melt test, so LP treatment required more temperature to be melted than the control.

Conclusions

LP solution was prepared using milk permeate powder and used successfully as an alternative to DSP in making PCF. This study concluded that as LP was used, the pH increased, which significantly impacted PCF properties. This study determined that LP could replace DSP to produce PCF with less melt and higher viscosity than DSP. The amount of LP can be adjusted based on the required functional characteristics of PCF. The LP has a significant impact on the physical and chemical properties of PCF. The study found that the PCF made with LP has more viscosity, hardness, melt temperature, and lower melting area.

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Tables

Table 1. Mean (n=5) of processed cheese food (PCF) formulations ($g.100g^{-1}$).

Treatments[1]	Ingredients						
	Cheddar cheese	**Water**	**Salted Butter**	**Deproteinized whey**	**Salt**	**Sodium phosphate dibasic**	**Lactose phosphate**
Control	66.84	17.01	3.50	8.15	2.0	2.5	-
LP	66.93	15.38	3.47	4.21	2.0	-	8.0

[1] Treatment: Control= Processed cheese food made with 2.5% disodium phosphate (DSP); LP= Processed cheese food made with 8% lactose-6-phosphate solutions

Table 2. Mean (n=5) composition of processed cheese food (PCF) made with either disodium phosphate (DSP) or lactose-6-phosphate solutions (LP).

Composition	Treatments[1]	
	Control	**LP**
Moisture (%)	42.61 [a]	43.09 [a]
pH	5.74 [a]	5.81 [b]

[1] Treatment: Control= Processed cheese food made with 2.5% disodium phosphate (DSP); LP= Processed cheese food made with 8% lactose-6-phosphate solutions

[a-b] Means in the same row not sharing a common superscript are different at $P<0.05$.

Table 3. Mean squares and P-values of pH and moisture of processed cheese food (PCF) with either Lactose-6-phosphate (LP) or sodium phosphate dibasic (DSP).

Factor	**df**	**Moisture**	**pH**
Replication	4	0.52 (0.40)	0.0015 (0.06)
Treatment	1	0.57 (0.30)	0.012 (<0.05)
Error	4	0.41	0.00028

Table 4. Mean (n=5) viscosity and hardness (g) of processed cheese food (PCF) with either Lactose-6-phosphate (LP) or sodium phosphate dibasic (DSP).

Parameter	Treatments[1]	
	Control	**LP**
Viscosity (cP)	1378 [a]	2032 [b]
Hardness (g)	91.61 [a]	154.55 [a]

[1] Treatment: Control= Processed cheese food made with 2.5% disodium phosphate (DSP); LP= Processed cheese food made with 8% lactose-6-phosphate solutions

[a-b] Means in the same row not sharing a common superscript are different at $P < 0.05$.

Table 5. Mean squares and P-values of end apparent cooked viscosity and hardness of process cheese (PCF) with either lactose-6-phosphate (LP) or disodium phosphate (DSP).

Factor	**df**	**End apparent cooked viscosity**	**Hardness**
Replication	4	20154.31 (0.78)	4597.18 (0.42)
Treatment	1	1069290 (<0.05)	9901.51 (0.18)
Error	4	46530.06	3781.13

Table 6. Mean (n=5) Schreiber melt test (mm) and melting temperature (°C) of processed cheese food (PCF) with different levels of lactose-6-phosphate (LP).

Parameter	Treatments[1]	
	Control	**LP**
Schreiber melt test (mm)	41.18 [a]	34.08 [b]
Melting temperature (°C)	43.23 [a]	46.58 [a]

[1] Treatment: Control= Processed cheese food made with 2.5% disodium phosphate (DSP); LP= Processed cheese food made with 8% lactose-6-phosphate solutions

[a-b] Means in the same row not sharing a common superscript are different at $P < 0.05$.

Table 7. Mean squares and P-values of Schreiber melt test (mm) and melting temperature (°C) of process cheese (PCF) with either lactose-6-phosphate (LP) or disodium phosphate (DSP).

Factor	**df**	**Diameter**	**DSR**
Replication	4	13.36 (0.16)	3.57 (0.91)
Treatment	1	126.17 ($P < 0.05$)	28.15 (0.26)
Error	4	4.61	17.001

Figures

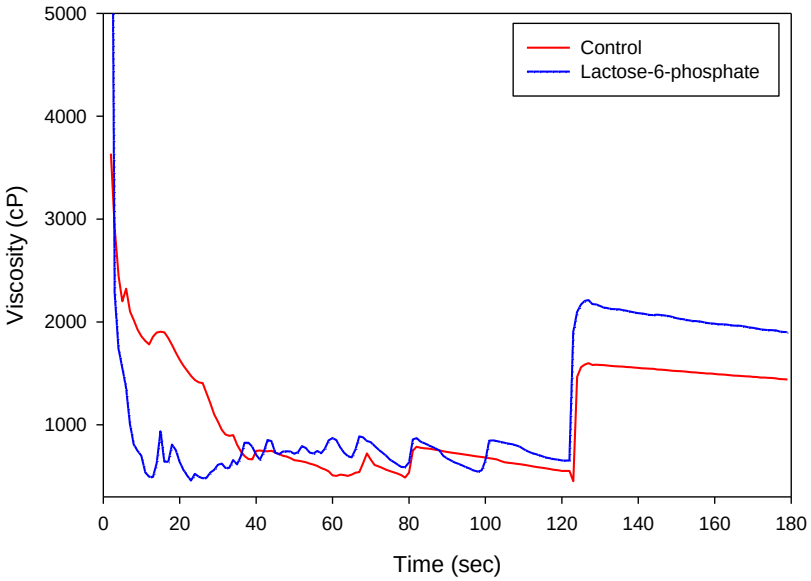

Figure 1. Measuring the apparent cooked viscosity of process cheese (PCF) using the Rapid visco analyzer (RVA).

Control= PCF made with 2.5% disodium phosphate (DSP); lactose-6-phosphate = PCF made with 8% lactose-6-phosphate solutions

CHAPTER VII: SUMMARY AND CONCLUSIONS

Lactose is used in food and pharmaceutical industries because it is a cheap ingredient, a source of low sweetness, and a filler. lactose-6-phosphate (LP) has been presented in milk and dairy products with low concentrations, changing some properties of dairy and food products. Thus, obtaining LP and studying its effect on processed cheese food (PCF) is highly desirable for the dairy and food industries. Not enough literature on LP is available, and there is a lack of information to understand the composition of this component. Additionally, there was no published data about using LP as emulsifying salts (ES) in PCF. The limitations of LP studies are as follow: i) Obtaining the right LP combination; ii) Finding pure lactose devoid of LP and other contaminants; iii) Directly analyzing LP and studying the emulsifying properties of LP.

Throughout the chapters of this dissertation, LP was prepared successfully using α-lactose monohydrate and milk permeate powder (MPP) in chapter 3. This study aimed to develop a method for phosphorylating α-lactose monohydrate (LaP1) and milk permeate powder (LaP2). Also, MS was used to define lactose and LP in α-lactose monohydrate, MPP, LaP1, and LaP2. MS was successfully used to determine the amount of lactose and LP. The remaining lactose in LaP1 and LaP2 decreased by about 77.52, and 49.51%, respectively. LP was increased after the phosphorylation process in LaP1 and LaP2. When comparing LaP1 and LaP2, we found a higher amount of LP in LaP1. This study concluded that the relative abundance of LP in MPP was higher than α-lactose monohydrate. During the phosphorylation process, the color of the solutions turns dark.

The dark color causes many challenges and limitations in using phosphorylation products. The activated carbon was successfully used to remove the dark color from LaP1 and LaP2 solutions reported in chapter 4. The compositional characteristics of the solutions, such as pH, total solids (TS), and color parameters (L*- lightness, a*- redness, and b*- yellowness) were examined at different stages (seven stages) of washing the solutions. Both solutions' pH and TS were significantly decreased with increasing the number of washings with activated carbon. When comparing parameters (L*, a*, and b*) of different stages, we found that the parameters a* and b* decreased but L* increased. ΔE decreased with increasing the number of stages for both solutions. We assume that activated carbon can be used to remove the dark color that results from the phosphorylation process.

One of the implications of this study was addressed in Chapter 5, where the LaP1 solution was prepared and used successfully as an alternative to ES, such as sodium phosphate dibasic (DSP), in making PCF. PCF was made using LaP1 solutions at different concentrations (2.0, 2.4, 2.8, 3.2, 4.0, 5.0, and 6.0%). PCF was made with 2.0% DSP as a control. When comparing PCF at the same pH and ES content, we assume that the higher the degree of casein dispersion caused by using a certain type of ES during cooking, the firmer the product after cooling. This study concluded that as LP concentration elevated, the pH increased, significantly impacting PCF properties. This study determined that LP could replace DSP to produce PCF with less meltability characteristics than DSP. The concentration of LP can be adjusted based on the required functional properties of PCF. The level of LP has a significant impact on the physical and

chemical properties of PCF. The study found that the higher the LP, the more significant viscosity, hardness, melt temperature, and lower melting area.

Chapter 6 reported that the LaP2 solution was prepared using MPP and used successfully as an alternative to DSP in making PCF. PCF was made using 8.0% LaP2 solutions and 2.5% DSP as a control. This study concluded that as LP was used, the pH increased, which significantly impacted PCF properties. This study determined that LP could replace DSP to produce PCF with less meltability and higher viscosity than DSP. The amount of LP can be adjusted based on the required functional characteristics of PCF. The LP has a significant impact on the physical and chemical properties of PCF. The study found that the PCF made with LP has more viscosity, hardness, melt temperature, and lower melting area.