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Influence of Transglutaminase and Thermal Treatment on Yield and Storage Stability of Traditional Palestinian Dairy Products

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Influence of Transglutaminase and Thermal Treatment on Yield and Storage Stability of Traditional Palestinian Dairy Products

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Thesis Approval

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Dedication

This thesis is dedicated to my lovely parents, my family, my close friends, who helped me to face difficulties during study and all research stages. This work dedicated also to my supervisor, my colleagues at Al-Quds and Hebron Universities, and to the dairy companies who interested in research and development in Palestine.

Hiba Ahmad Mahmod Abed

Declaration:

I certify that this thesis submitted for the degree of master is the result of my own research, except where otherwise acknowledged, and that this thesis (or any part of the same) has not been submitted for a higher degree to any other university or institution.

Signed: Hiba

Hiba Ahmad Mahmod Abed

Date: 07-08-2023

Acknowledgement

Praise and thanksgiving always and forever to God for the blessings of giving, conciliation, patience and success.

Firstly, I should express my due thanks and gratitude to my mentor and supervisor Dr. Ziad Ayyad for his advice, guidance, assistance, and giving me a lot of his time for applying experiments, making creative discussion and providing developmental and constructive suggestions.

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My heartfelt thanks extended to my parents, for providing me with all kinds of support and encouragement to complete this dissertation.

To my family, my close friends, my colleagues, and all of those who gave assistance, helped me, shared with me by any and every way, to all of them I say: thank you so much.

Hiba A Abed

Abstract

In the conventional dairy processes, a considerable amount of protein is lost with the whey, as protein is a valuable component of milk and milk products, therefore, incorporating whey proteins in dairy products will increase the yield, as the presence of whey proteins allowed higher moisture retention, due to their hydrophilic property, and thus higher yield obtained.

Several methods have been used to reduce the whey separation in dairy products, such as application of stabilizers, milk derived constituents, and starter cultures. One of the methods that also used to reduce loss of whey protein is the enzymatic treatment of milk with Transglutaminase. TG enzyme form new intra- and intermolecular crosslinks between milk proteins. Such crosslinks can modify the structure and functionality of the proteins.

Several factors affect the efficiency of Transglutaminase whey proteins crosslinking with curd proteins. One of the most important factors is heat treatment of milk. Thus, this study aimed to investigate the influence of different pasteurization temperatures combined with different enzyme concentrations, on the yield and whey percentages in the concentrated yogurt (Labneh), and in the yield percentage of the local white- brined cheese.

Concentrated yogurt (Labneh) milk was pasteurized at different pasteurization temperatures (65 C°/ 30 min, 72 C°/ 15 sec, 82 C°/ 15 sec, and 90 C°/ 15 sec), and after each pasteurization, the milk was treated with different TG ratios (0, 1, 3, and 5 gm TG/kg milk). As well as, in the local white- brined cheese manufacturing, milk was pasteurized at different pasteurization temperatures (65 C°/ 30 min, 72 C°/ 15 sec, and 82 C°/ 15 sec), and after each pasteurization, the milk was treated with different TG ratios (0, 1, 3, and 5 gm TG/kg milk).

This study demonstrated that the inclusion of TG into the concentrated yogurt increases yield percentage, and reduces whey loss. In addition, TG inclusion into the local white brined cheese increases the yield percentage. The obtained yield improvement was due to TG crosslinking of milk proteins. Results showed that the concentrated yogurt yield significantly improved when the milk was pasteurized at (90 C°/ 15 sec), followed by treatment with enzyme ratio of (3 gm TG/ kg milk). For the local white-brined cheese, yield significantly improved when the milk was pasteurized at (72 C°/ 15 sec), and followed by treatment with enzyme ratio of (3 gm TG/ kg milk).

Concentrated yogurt (Labneh) samples with highest yield and lowest whey percentages, as well as, the local white brined cheese samples with highest yield percentage, underwent physicochemical and microbial analysis when fresh, at (15, and 30) day of storage period.

The physicochemical results for both products showed that samples produced with enzyme treatment exhibited higher pH values, lower titratable acidity values, higher moisture content, lower syneresis (for treated Labneh samples), and lower dry matter content (for treated cheese samples). In terms of microbial analysis, results showed that in treated Labneh samples enzyme crosslinking retard the growth of lactic acid bacteria resulting in lower numbers and longer time of coagulation. In treated local white- brined cheese samples, coliform counts were lower than the control during storage period. However, both TG treated products exhibited higher yeast and mold growth than the control samples.

The study recommended mainly to use TG as method to improve the yield, especially if combined with the suitable pasteurization temperature for each product. In addition to the possibility of dispensing the stabilizers use. It also recommends further studies to investigate the sensorial and rheological properties of TG treated samples. As well, studies needed to investigate the effect of transglutaminase addition on the fermentation or coagulation time; to take time cost into consideration.

تأثير الترانسجلوتاميناز والمعاملة الحرارية على الإنتاج وثباتية التخزين لمنتجات الالبان الفلسطينية التقليدية

اعداد: هبة أحمد محمود عابد

اشراف: د. زياد عياد

ملخص

في عمليات الألبان التقليدية، يتم فقدان كمية كبيرة من البروتين مع مصل اللبن، حيث يعتبر البروتين مكونًا مهمًا في الحليب ومنتجات الألبان، وبالتالي فإن دمج بروتينات مصل اللبن في منتجات الألبان سيزيد من الانتاج، حيث يسمح وجود بروتينات مصل اللبن بدرجة أعلى الاحتفاظ بالرطوبة، بسبب خصائصها المحبة للماء، وبالتالي الحصول على إنتاجية أعلى.

هنالك عدة طرق مستخدمة لتقليل فصل مصل اللبن في منتجات الالبان، مثل استخدام المثبتات، والمكونات المشتقة من الحليب، والبادئة. واحدة من الطرق المستخدمة أيضا لتقليل فقد بروتين مصل اللبن هي المعالجة الإنزيمية للحليب باستخدام انزيم ترانسجلوتاميناز . يشكل إنزيم ترانسجلوتاميناز روابط متقاطعة جديدة

داخل وبين بروتينات الحليب. يمكن لمثل هذه الروابط المتقاطعة تعديل بنية ووظيفة البروتينات.

عدة عوامل تؤثر على كفاءة ربط بروتين مصل اللبن ببروتينات الخثارة بفعل انزيم ترانسجلوتاميناز. من أهم هذه العوامل هي المعالجة الحرارية للحليب. وبالتالي، هدفت هذه الدراسة إلى معرفة تأثير درجات حرارة البسترة المختلفة المقترنة بمعاملة الحليب بتراكيز مختلفة من انزيم ترانسجلوتاميناز، على نسبة الانتاج ونسبة مصل اللبن من اللبن المركز (اللبنة)، ونسبة الإنتاج للجبنة البيضاء المحلية المحفوظة بمحلول ملحي.

تم بسترة حليب لبن الزبادي المركز (اللبنة) بدرجات حرارة مختلفة (٦٥ درجة مئوية / ٣٠ دقيقة، ٧٢ درجة / ١٥ ثانية، ٨٢ درجة / ١٥ ثانية، ٩٠ درجة / ١٥ ثانية)، وبعد كل بسترة، تمت معالجة الحليب بنسب مختلفة من انزيم ترانسجلوتاميناز (٠، ١، ٣، ٥ غرام انزيم / كجم حليب). بالإضافة إلى ذلك، في صناعة الجبنة البيضاء المحلية، تمت بسترة الحليب في درجات حرارة مختلفة (٦٥ درجة مئوية / ٣٠ دقيقة، ٧٢ درجة مئوية / ١٥ ثانية، ٨٢ درجة مئوية / ١٥ ثانية)، وبعد كل بسترة، تم معالجة الحليب بنسب مختلفة من انزيم ترانسجلوتاميناز (٠، ١، ٣، ٥ غرام انزيم / كغ حليب).

أظهرت هذه الدراسة أن تضمين انزيم ترانسجلوتاميناز في اللبن المركز (اللبنة) يزيد من نسبة الانتاج ويقلل من فقد مصل اللبن. بالإضافة الى ذلك، يؤدي تضمين انزيم ترانسجلوتاميناز في الجبنة البيضاء المحلية إلى زيادة نسبة الانتاج. التحسن في نسبة الإنتاج كان بسبب ربط بروتينات الحليب بفعل نشاط انزيم الترانسجلوتاميناز. أظهرت النتائج أن نسبة انتاج الزبادي المركز (اللبنة) تحسنت عندما بستر الحليب عند (٩٠درجة مئوية / ١٥ ثانية)، تبع ذلك معالجة بالأنزيم بنسبة (٣ جم انزيم / كجم حليب). بالنسبة للجبنة البيضاء المحلية المحفوظة بمحلول ملحي، تحسنت نسبة الانتاج بشكل كبير عندما بستر الحليب عند (٧٢درجة مئوية / ١٥ ثانية)، تبع ذلك معالجة بالأنزيم بنسبة (٣ جم انزيم / كجم حليب).

عينات اللبن المركز (اللبنة) ذات أعلى إنتاج وأقل نسب مصل اللبن، وكذلك عينات الجبنة البيضاء المحلية المحفوظة بمحلول ملحي ذات أعلى نسبة إنتاج، خضعت لتحليل فيزوكيميائي وميكروبي عندما تكون طازجة وعند (١٥، و٣٠) يومًا من فترة التخزين.

أظهرت النتائج الفيزوكيميائية لكلا المنتجين أن العينات المنتجة بمعالجة الترانسجلوتاميناز أظهرت قيمًا أعلى للأس الهيدروجيني، وقيم حموضة أقل، ومحتوى رطوبة أعلى، وفقدان مصل أقل (لعينات اللبنة المعالجة)، ومحتوى أقل

من المادة الجافة (لعينات الجبنة المعالجة). على صعيد التحليل الميكروبي، أظهرت النتائج أنه في عينات اللبنة المعالجة، أدى ربط بروتينات الحليب بواسطة انزيم الترانسجلوتاميناز الى تأخير نمو بكتيريا حمض اللاكتيك، وبالتالي اعداد اقل ووقت تخثر اطول. في عينات الجبنة البيضاء المحلية المعالجة، كان تعداد البكتيريا القولونية أقل من عينة التحكم خلال فترة التخزين. بينما كلا المنتجين المعالجين بالأنزيم أظهروا نموًا في الخميرة والعفن أعلى من عينات التحكم.

أوصت الدراسة بشكل أساسي باستخدام انزيم ترانسجلوتاميناز كطريقة لتحسين الانتاج، خاصة إذا تم دمجه مع درجة حرارة البسترة المناسبة لكل منتج. بالإضافة إلى تحسين الإنتاج، إمكانية الاستغناء عن استخدام المثبتات. كما أوصت الدراسة بإجراء مزيد من الدراسات للتحقيق في الخصائص الحسية والانسيابية للعينات المعالجة. كذلك، يلزم إجراء دراسات لاستقصاء تأثير إضافة ترانسجلوتاميناز على فترة التخمير أو التخثر؛ لأخذ تكلفة الوقت في الاعتبار.

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Abbreviations

BAM: Bacteriological Analytical Manual.

CMP: Caseinomacropeptide.

DM: Dry Matter.

FDA: Food and Drug Administration.

HT: Heat Treatment.

LAB: Lactic Acid Bacteria.

LMW Peptides: Low Molecular Weight Peptides.

MTGase: Microbial Transglutaminase Enzyme.

PCBS: Palestinian Central Bureau of Statistics.

PSI: Palestinian Standard Institution.

TA: Titratable Acidity.

TBC: Total Bacterial Count.

TC: Total Coliform.

TG- WBC: Transglutaminase treated white- brined cheese.

TG: Transglutaminase Enzyme.

WBC: White- Brined Cheese.

WHC: Water Holding Capacity.

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CHAPTER ONE

INTRODUCTION

Chapter One Introduction

1. Introduction:

1.1. Milk Products

Milk can be defined as the secreted fluid of the mammary glands of female mammals. Secreted in the mammary glands as a natural process after parturition of the newborn. It contains nearly all the nutrients necessary to sustain life (Bateman et al., 2006). The main components of liquid milk are water, proteins, lipids, carbohydrate merely (lactose), vitamins, and minerals (Vaclavik et al., 2008; El Kiyat et al., 2021).

According to legislations, milk and milk products must contain a designated percent of total milk solids (all of the components of milk except water). Cow's milk consists of about (87%) water, and (12–13%) total solids. The solids consist of fat (~4%) and solids-not-fat (SNF) (~9%), such as proteins, lactose, and various minerals and vitamins (Burke et al., 2018). The butterfat component of milk is the most expensive component of milk and its level determines if milk is offered for retail sale as whole milk or low fat milk, such as 2% fat, 1%, 1/2%, or fat-free (Vaclavik et al., 2008).

Milk products such as yogurt, cheese and kefir with different taste, texture, nutritive value, and shelf life are produced by milk fermentation, coagulation, drying, homogenizing, or pasteurizing (El Kiyat et al., 2021). Milk processing provide a wide variety of products such as dried milk powder, which is added to a multitude of foods to increase the protein or calcium value, cream, ice milk, ice cream, sour cream, yogurt, and cheeses (Vaclavik et al., 2008).

Yogurt promote health benefits make it widely produced and well accepted by consumers (Widyastuti and Febrisiantosa, 2013). Yogurt goes through different
processes to create different yogurt products. Processes include freezing to produce frozen yogurt, whipping to produce yogurt butter or ghee, other processing include churning with water to produce drinking yogurt (Tamime and Robinson, 2000).

Concentrated yogurt, known as Labneh, which is a semi solid food product derived from yogurt by straining a part of its water and water soluble compounds (Tamime and Robinson, 1999). Cloth or paper bag are used as filters to remove the whey, giving a consistency between that of yogurt and cheese, while preserving yogurt's distinctive sour taste. Like many yogurts, strained yogurt is found originally in Middle East area, especially in Lebanon, Jordan, Morocco and Iraq (Basiony et al., 2017).

In Syria and Jordan, Labneh is manufactured from the fermented milk called Laban which is normally made from ewe's milk. As the ewe's milk is seasonal, so the people manufacture Laban and turn it into Labneh, which is stored in olive oil for more than one year. In Western Europe and in the U.S., strained yogurt has become increasingly popular because it is richer in texture than unstrained yogurt (Basiony et al., 2017).

Cloth-bag prepared Labneh, produced by Cattle milk pasteurization, cooling to proper incubation temperature, followed by inoculating starter culture which are traditionally composed of (Streptococcus thermophilus and Lactobacillus bulgaricus). After addition of starter culture in proper amount, the product then held for about (~4- h) until (pH~ reach 4.6). Then the resulting yogurt curd, placed in cloth bags and left to drain by gravity at (6°C) overnight (Yamani and Abu-Jaber, 1994). The obtained

Labneh is a white to creamy paste that has a smooth texture, with a taste crossing between sour cream and cottage cheese (Varman and Sutherland, 1994; Tamime and Robinson, 1999). Labneh flavour is slightly acidic, however, perception of low acidity stems from the masking effect of the high fat content of Labneh, which is around (10 g 100 g-1). Moreover, titratable acidity may be within the range of (1.8–2.0%) as calculated as lactic acid (Rasic, 1987).

Cheese considered as one of the excellent sources of calcium and its health benefits extended to maintain healthy bones and teeth. Beside the nutritional value that are obtained from conserving the nutritious component of milk by the cheese making process, it also aimed to extend the shelf life. However, besides that purposes, recently there is a growing interest to develop a variety of cheese products with other advantages, such as improving and diversifying the taste or flavor of cheese products to fit the consumer's choice (Widyastuti and Febrisiantosa, 2013).

Cheese is produced by separating milk into solid curds and liquid whey by coagulation (Afandi et al., 2013). During cheese production, casein, the main milk protein, is coagulated using proteolytic enzymes. Rennet one of the most commonly used enzymes in cheese production, it comprises a mixture of enzymes and is produced in the stomach of ruminant mammals. Chymosin is the key component of rennet that curdles milk casein, which then become insoluble in water and allows it to separate from the solution (Miskiyah et al. 2011; Ozcan and Eren –Vapur, 2013).

Rennet coagulation of milk is the first step in cheese production. The cheese curd is formed of caseins, which represent about (80%) of total milk proteins, while soluble proteins are mostly lost in the whey (Britten and Giroux, 2022).

The rennet coagulation of milk is a two-step process. The primary phase of coagulation corresponds to the enzymatic hydrolysis of the κ-casein present at the surface of casein micelles (Corredig & Salvatore, 2016; Horne & Lucey, 2017). The C-terminal region of the κ-casein is mostly hydrophilic with a net negative charge at native milk pH (pH ~6.7). It protrudes from the micelle surface, forming a hairy layer (Dalgleish & Corredig, 2012). This layer provides stability to the micelles through steric hindrance and electrostatic repulsion.

The chymosin from the rennet cleaves the κ-casein at the (Phe105–Met106) position resulting in the release of caseinomacropeptide (C-terminal fragment 106–169) in the serum phase of milk. It has been observed that the diameter of the casein micelles decreases by about (10 nm) following the cleavage of κ-casein by rennet (Sandra et al., 2007). The para-κ-casein (N- terminal fragment 1–105) is positively charged at milk pH (pH ~6.7) and remains attached to the casein micelles.

The secondary phase of coagulation consists in the aggregation and gelation of the destabilized micelles (Horne & Lucey, 2017). When about (80% to 90%) of κ-casein is hydrolyzed (McSweeney, 2007), the micelles begin to aggregate, via hydrophobic interactions and calcium bridges, forming a gel network. The pores of the gel are relatively large and contain whey and fat globules.

Cheese is considered the most widely made dairy product in many varieties. (Eekles et al., 1951) claimed that there are about (18) distinct varieties of cheese with more than (400) commercial names according to country of production.

White cheese is produced globally in almost (1000 to 4000) varieties (Sert et al., 2007), under names such as Feta in Greece, Domiati in Egypt, and Beyaz peynir (white cheese) in Turkey (Alichanidis & Polychroniadou, 2008). The advent of white cheese dates to around (1400 AD) when they were made primarily by the Turks. Until the (1950s), white cheese was mainly produced only by village families; it was only in the (60s) that variety of cheese arrived in the major cities (Kamber, 2007).

In Palestine, the dairy sector occupies the largest market share in the local market by a percentage of (80%). Where (41) factories registered in the Ministry of National Economy work in this sector, and these factories employ about (3) thousand workers. According to the statistics of the General Federation of Food Industries, the production capacity of dairy factories ranges from (550-600) tons per day and covers basic commodities such as Labneh, yoghurt and white cheese (The Palestinian Information center, 2018).

1.2. Nutritional Value of Milk and Milk Products

The contributions of milk components and dairy products to human health have been comprehensively reviewed by (Tunick and Van Hekken, 2015). The health benefits can be summarized as enhancing muscle building, lowering blood pressure, reducing low density lipoprotein cholesterol, and preventing diabetes, obesity and cancer, among others. (Thorning et al., 2016) who found that the dairy products intake might improve body composition, and it was associated with reduced risk of type (2) diabetes, a reduced risk of cardiovascular disease (e.g., stroke), and a reduced risk of colorectal cancer, bladder cancer and breast cancer. (Vargas-Bello-Pérez et al., 2019) showed that the milk proteins and their fractions are the main source of bioactive peptides, which are considered as potential ingredients for health promoting functional foods.

In addition to the nutritional importance of milk and milk derivatives, the dairy industry contributes significantly in raising the economic value of the country food industries.

1.3. Economic Value of Milk and Milk Products in Palestine

The value of dairy industries production amounted to ($153.5) million, which represent (17%) of the food industries in (2018). ($128.1) million of which are in the West Bank, represent about (83.4%) of the production of the dairy industry. and ($ 25.4) million in the Gaza Strip account for about (16.6%) of the dairy industry (PCBS, 2020).

1.4. Transglutaminase Enzyme

In the conventional dairy processes, a considerable amount of protein is lost with the whey, as protein is a valuable component of milk and milk products, therefore, incorporating whey proteins in dairy products will increase the yield, as the presence of whey proteins allowed higher moisture retention, due to their hydrophilic property, and thus higher yield obtained (Meinardi. et al., 2003). Increasing yield accompanied by increasing the profit – especially if the whey incorporating process can be adapted without proportional expenses, e.g. expensive equipment-, and will reduce the expenditure on wastewater treatment (Jensen and Stapelfeldt, 1993).

Several methods have been used to reduce the amount of whey separation - such methods include the application of stabilizers, milk-derived constituents and starter cultures (Ladjevardi, et al, 2015; And &Guo, 2006). On the other hand, whey protein can be recovered by concentration or drying the whey proteins and adding it to the dairy curd, heat or pressure treatment of the milk, and concentration of the milk by ultrafiltration, evaporation or reverse osmosis (Ernstrom et al., 1980;

Hinrichs, 2001; Kosikowski and Mistry, 1977; Lo and Bastian, 1998; O'Reilly et al., 2001).

One of the most attractive methods of recovering whey proteins is the enzymatic treatment of milk or curd with transglutaminase which links whey proteins with curd proteins leading to a noticeable increase in protein and yield of the dairy product, accompanied by a decrease in whey protein content (Mahmood and Sebo, 2009). This treatment has economical, nutritional and environmental importance.

Enzymatic cross-linking received increasing attention during the last (10) years (Motoki and Seguro, 1998; Faergemand et al., 1997; Faergemand et al., 1999). So, recently, instead of addition of extra protein, and polysaccharide- based stabilizers, enzymatic modification of milk proteins has been exerted to improve the quality of dairy products (Gharibzahedi and Chronakis, 2018).

Transglutaminase enzyme (TG) was initially identified by Heinrich Waelsch since more than (40) years ago as a liver enzyme incorporating amines into proteins (Fesus and Piacentini, 2002). In the early (1980s), the possibility of modification of functional properties of milk caseins and soybean globulins was demonstrated using TG derived from guinea pig liver or bovine plasma (Motoki and Seguro, 1998).

The limited supply of guinee pig liver and the social and technical barriers against its use in food applications have encouraged researches to find alternative sources for TG, such as microorganisms. The TG-like enzymes secreted by the microorganisms were screened for their ability to form glutamine-lysine bonding between food proteins, as a characteristic linked to TG (Nonaka et al., 1989). TG enzyme was extracted from a variant of Streptoverticillium mobaraense which found to be suitable for food applications (Washizu et al., 1994). Later, other microorganisms were also used for production of microbial TG (MTGase).

Since (1998), TG with “No. GRN 000095” was approved by the FDA as “Generally Recognized as Safe (GRAS)” (FDA., 2001). There are confirmed documents for MTGase in the US, Japan and Europe as a safe ingredient in different types of processed foods (Gerrard et al., 2000; Gharibzahedi and Chronakis ,2018; Gharibzahedi et al., 2019).

Transglutaminase (EC 2.3.2.13) forms covalent crosslinks of inter- or intra-molecular ε-(γ glutamine)-lysine isopeptidic bonds by catalyzing an acyl transfer reaction between a γ-carboxyamide group in protein-bound glutamine residues (acyl donor) and an ε-amino group in a protein-bound lysine residue (acyl acceptor) (Seguro et al., 1996). This will lead to the formation of new intra- and intermolecular crosslinks. Such crosslinks can modify the structure and functionality of proteins, and improves the yield and water-holding capacity (Romeih and Walker, 2017). The TG-catalyzed reactions are schematically shown in Figure 1.1 (Zhu et al. 1995; Motoki and Seguro 1998; Sharma et al. 2001).

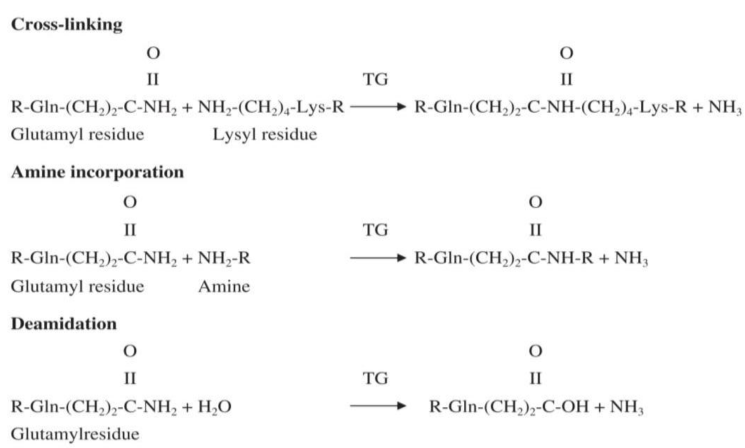

Figure 1.1 TG-catalyzed reactions.
Source: (Zhu et al. 1995; Motoki and Seguro 1998; Sharma et al. 2001).

This enzyme works optimally at a pH of (5– 8), but still shows enzymatic activities at pH range from (4 to 9) with a specific activity of (22.6 U/mg) (Motoki and Seguro 1998). Moreover, transglutaminase remain active and stable between pH (5 and 9), the range considered suitable for most food products (Martins et al., 2014). Nevertheless, the optimal temperature of transglutaminase activity is (45– 50°C) (Özer et al., 2013).

Several factors may affect the efficiency of transglutaminase in stimulating the crosslinking of whey proteins with the curd proteins such as temperature, pH, concentration of the enzyme, availability of cofactors and milk heat treatment (Mahmood & Sebo, 2009).

Heat treatment is the most widely used processing technology in the dairy industry. Such treatments aimed to destroy microorganisms, both pathogenic and spoilage, to ensure the milk is safe and has a reasonable shelf-life (Deeth, 2021).

Heat treatment would modify the protein structural organization, and inactivate some enzymes (Lucey et al., 1998) and it also expected to cause a certain degree of protein denaturation, which would benefit the gel formation (De Brabandere & De Baerdemaeker, 1999).

The high level of flexibility of casein proteins, along with their little or no secondary protein structure, makes them easier to be cross-linked by TG, in comparison to the whey protein with a globular dense structure (El-Kholy, 2005). The globular whey proteins stabilized by disulfide bridges (Sharma et al., 2002). Therefore, whey protein requires steps that promote their partial denaturation to make them accessible to the enzymatic activity (DeJong and Koppelman, 2002; Bonisch et al., 2007).

Heat treatment induces TG- reaction, by increasing whey protein susceptibility to TG crosslinking. Whey proteins are denatured by the unfolding of their polypeptides, thus exposing the side chain groups originally buried within the native structure (Singh and Waungana, 2001). The unfolded proteins then, interact with casein micelles or simply aggregate with themselves. In the presence of TG enzyme, increasing degree of whey protein denaturation by heat increases the protein polymerization that is triggered by TG (Lorenzen, 2000a).

1.5. Problem Statement

The problem of the study is represented as follows:

1.5.1. The absence of standardized manufacturing methods for concentrated yogurt (Labneh) and local white- brined cheese, in terms of production line settings, specifically thermal treatments. Where the pasteurization temperature of a specific product varies from one facility to another based on the efficiency of the pasteurizer used, the scientific background of the facility owner or the pasteurizer operator, or special experiments that reflected satisfactory microbial and productivity results.

1.5.2. Whey protein loss in commercial manufacturing methods, which considered as nutritional, environmental, and economical problem. Particularly during straining or pressing of the Labneh and cheese products. Whey loss constitutes a waste that increases the financial burden of treating waste water, in addition to that the less whey loss, the more effects on the yield percentage achieved and, accordingly, the gained profit.

1.5.3. The limitation in the use of stabilizers to reduce the whey loss, in terms of the type and the use extent. For example, it is forbidden to use starch and fillers such as flour or gelatin according to Palestinian legislations (PSI, 2020,2016).

1.5.4. There are limited studies that investigate the effect of different pasteurization temperatures and milk treatment with different transglutaminase ratios to increase yield of concentrated yogurt (Labneh) and white brined cheese, as a result of TG crosslinking.

1.6. Study Hypothesis

The final stage of concentrated yogurt (Labneh) production as well as white cheese includes removal of whey from the product. This is done by Labneh straining and also White- brined cheese pressing. Thus, both products have a percentage of whey loss that affects the yield percentage. Whey loss percentage is related to the manufacturing parameters and the added stabilizers. So, it is assumed that the link between the optimum pasteurization temperature and the optimum transglutaminase concentration for each product, will greatly affect the yield percentage for both Labneh and white- brined cheese, since both factors have a direct effect on milk proteins (casein and whey).

1.7. The Research Objectives

The research main objective:

Investigate the influence of Transglutaminase and thermal treatment on the yield and storage stability of concentrated yogurt (Labneh), and local white- brined cheese.

The main objective will be achieved by achieving the following sub-objectives:

1.7.1. Investigate the influence of different pasteurization temperatures combined with different transglutaminase treatments of milk on the concentrated yogurt yield and whey percentages (Syneresis %).

1.7.2. Investigate the influence of different pasteurization temperatures combined with different transglutaminase treatments of milk on the white- brined cheese yield percentage.

1.7.3. Determining the optimal combination of pasteurization temperature and TG enzyme ratio, resulting in the highest yield and the lowest whey percentages in concentrated yogurt (Labneh) production.

1.7.4. Determining the optimal combination of pasteurization temperature and TG enzyme ratio, resulting in the highest yield percentage in white brined cheese production.

1.7.5. To follow the quality of the highest yield samples (for both concentrated yogurt –Labneh-, and white- brined cheese) in terms of physicochemical and microbial characteristics, during a month of storage, and comparing them with control samples to evaluate and interpret the changes in products properties.

1.8. Summary of Experimental Investigations

This research will investigate the influence of different pasteurization temperatures combined with different transglutaminase treatments for milk, on the yield percentage of two different products: concentrated yogurt (Labneh), and white- brined cheese. The highest yield samples (result from the optimum combination of pasteurization temperature and TG concentration), and control sample for both products subjected to different physicochemical and microbial analysis during storage period (Fig 1.2, and Fig.1.3).

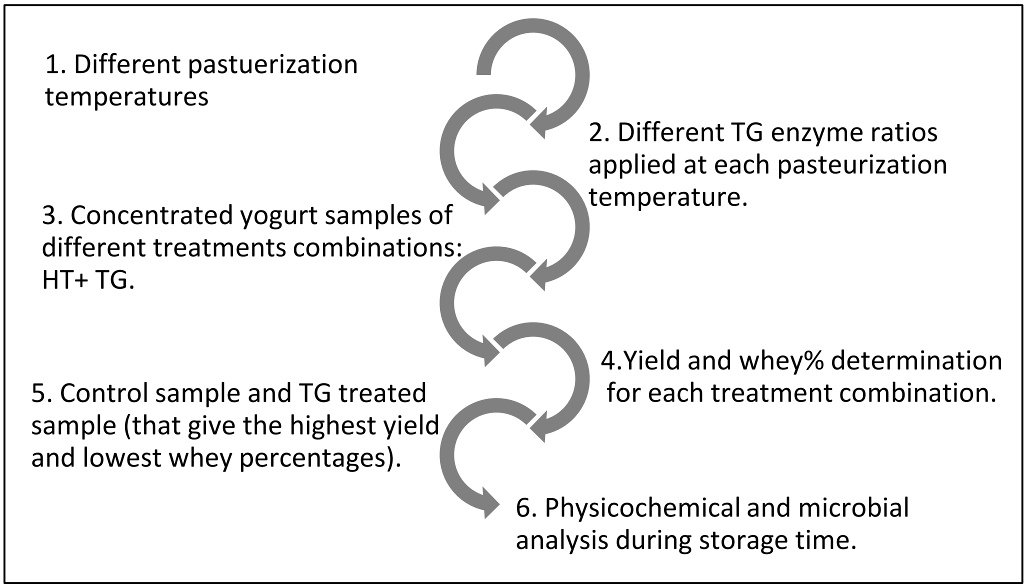

Fig.1.2: Representative chart for the concentrated yogurt (Labneh) experimental work.

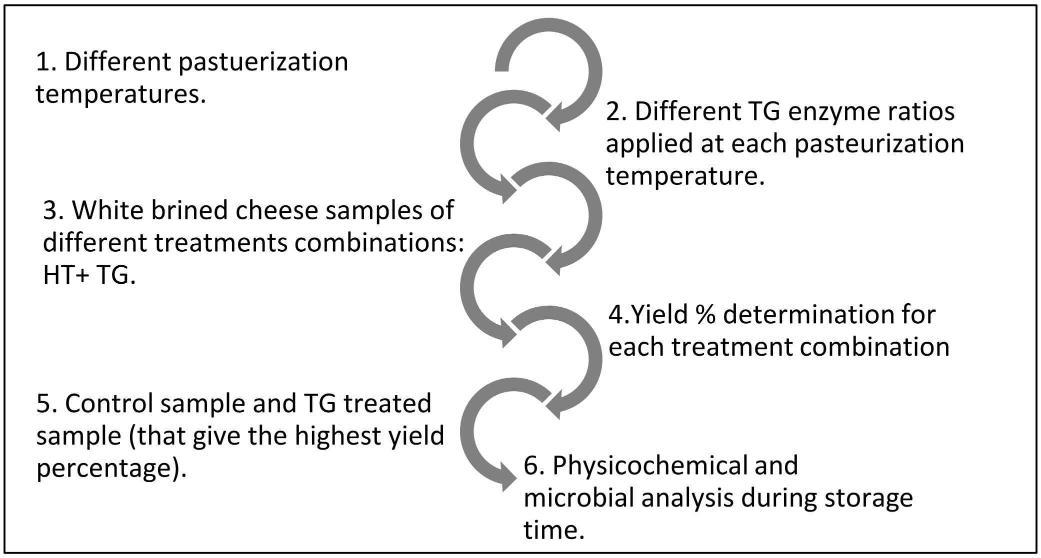

Fig.1.3: Representative chart for the local white- brined cheese (WBC) experimental work.

Al-Quds University

CHAPTER TWO

LITERATURE REVIEW

Chapter Two Literature Review

2.1. Introduction

This chapter will primarily focus on previous studies carried out on dairy products to investigate the effect of TG enzyme addition, studies carried out to investigate the factors that affect TG- crosslinking of milk proteins, and that investigate the influence of Transglutaminase cross-linkage on fermentation time and rennet clotting Time.

2.2. Crosslinking of Milk Proteins by Transglutaminase Enzyme.

Between the two main protein fractions of milk, the caseins can be easily cross-linked by MTGase, whereas the globular whey proteins are hardly susceptible to MTGase-induced reaction (de Jong and Koppelman, 2002; Lorenzen, 2002).

For casein micelles, the increase in polymerization could be traced back to a more open structure of the casein micelle associated with better MTGase accessibility (Bönisch et al. 2004).

Casein is considered as a good substrate for MTGase action due to its open tertiary structure (Christensen et al, 1996; Faergemand and Qvist, 1997). However, Tolkach and Kulozik (2005) mentioned the high availability of κ-casein to the MTGase action because it possesses four active glutamine residues, two of which are present in the hydrophilic caseinomacropeptide (CMP) region which are susceptible to cross-linking.

The globular whey proteins are hardly susceptible to MTGase, but the accessibility of β-lactoglobulin and α-lactalbumin to MTGase-catalyzed reaction can be enhanced by reducing agents such as DTT reducing agent, pH increase, heat treatment, and high hydrostatic pressure. DTT (Dithiothreitol) was found to cleave disulfide bonds, leading to unfolding of the protein and resulting in an exposure of potential new cross-linking sites for MTGase (Coussons et al. 1992; de Jong and Koppelman, 2002). Besides reducing agents such as DTT, an increase in pH can also affect the molecular structure of proteins. β-lactoglobulin is partially unfolded at pH (8.5–9.0); at this particular pH range, MTGase is still active, and the protein can be polymerized (Faergemand and Qvist, 1998). Furthermore, heat treatment (O'Sullivan et al., 2002) and the application of high hydrostatic pressure (Lauber et al., 2003) induce a denaturation of β-lactoglobulin, which results in a better accessibility for MTGase.

2.3. Applications of Transglutaminase on Different Dairy Products.

TG enzyme has been approved by the food industry to improve quality of many foods such as meat, fish, soy products, yogurt, ice-cream, and cheese (Zhu et al., 1995; Motoki and Seguro, 1998; Kuraishi et al., 2001).

Several studies have focused on the application of transglutaminase enzyme (TG) in dairy products.

TG application in dairy products increases the possibility of gel strength, water holding capacity, stability, and mechanical properties (Özrenk, 2006).

Seguro et al. (1996) found that TG cross-linkage change the protein structure and improve its functional properties, like texture, viscosity and water holding capacity without affecting the nutritional quality of the lysine residue.

Cozzolino et al. (2003) concluded that when the MTGase enzyme was added after the enzymatic coagulation stage of cheese milk, the resulting cheese exhibited higher protein content, denser curds, greater hardness and higher deformability than the control (without MTGase enzyme treatment), and that the yield was increased proportionally to the decreased amount of whey loss.

Farnsworth et al. (2006) Showed that TG cross-linking significantly enhance gel consistency, and reduce whey separation of goat milk yogurt, when (0.5%) of TG enzyme was used.

Piccolo (2006) studied the effect of adding MTGase (100 U. g^{-1}) on the cottage cheese making process, the study observed that, a (13%) increase in yield after MTGase Enzyme treatment, compared with control samples.

Özer et al. (2007) concluded that, the higher concentrations of TG reduced syneresis, along with increase in the yogurt viscosity. However, TG might have a slight adverse effect on the growth of yogurt starter bacteria causing slower development of acidity and acetaldehyde production compared with the control yoghurt sample.

Bönisch et al. (2008) reported that, transglutaminase can increase the yield, and improve the texture of cheese products without adding other chemicals.

Faria (2010) revealed that, fermented whey drink treated with MTGase showed a lower degree of syneresis after (14) days of storage with respect to control sample without MTGase enzyme. This study also observed that the viscousness of the milk beverage increased with increasing concentrations of MTGase enzyme.

Aprodu et al. (2012) reported that the enzyme increased gel strength, and decreased syneresis of yogurt.

Mazuknaite (2013) found that the enzyme gave higher gel firmness, increased the yield, decreased syneresis and improved organoleptic properties of soft cheese.

Sayadi et al. (2013) revealed that, addition of transglutaminase and rennet enzymes increase the yield of low-fat cheese compared with the control (9.2%±0.28% vs 6.5±0.45%). The yield was enhanced by increasing the serum proteins bonding within the gel network.

Aaltonen et al. (2014) reported that transglutaminase significantly increased the yield of Edam cheese by binding additional water molecules without reducing the hardness of the cheese due to the crosslinking of protein molecules.

Sumarmono. et al (2019) investigated the yield and processing properties of concentrated yogurt manufactured from local cow's milk with the addition of microbial transglutaminase enzyme (MTGase) and several thickening agents. The study showed that the yield and the processing properties of concentrated yogurt can be improved by the addition of enzyme (MTGase) and thickening agents such as inulin, carrageenan, xanthan, and pectin. The use of xanthan resulted in the highest yield, whereas the use of inulin and MTGase produce yogurt curd with low syneresis as well as high-water holding capacity.

2.4. Factors affect TG- Crosslinking of Milk Proteins.

2.4.1. Reaction Temperature.

Ando et al. (1989) determined an optimum reaction temperature of

(50C°) at a pH of (6.0). The enzyme retained some residual activity near the freezing point but, at (70C°), lost its activity within a few minutes (Seguro et al. 1996; Yokoyama et al. 2004).

Tsai et al. (1996) found that the optimal temperature for enzyme reaction was (50C°).

Motoki and Seguro (1998) reported that the enzymatic activity of transglutaminase is optimum at a temperature range of (50–55C°), and the enzyme shows continuous activity at (50C°) for (10) min. Transglutaminase can even work at temperatures as low as (10C°), and some enzymatic activities are retained even at temperatures above freezing. A previous study reported that the optimal temperature of transglutaminase activity is (45– 50C°) (Özer et al. 2013).

2.4.2. Reaction pH.

The isoelectric point of MTGase is (8.9), and the optimum pH ranges between (6.0 and 7.0), with some residual activity at pH (4.0 and 9.0) (Ando et al. 1989).

In skimmed and reconstituted milk, transglutaminase has an optimal pH of (7.0–7.5) (Hinz et al. 2012). At pH (6.6), the enzymatic reaction begins earlier in the process (Bönisch et al. 2007a).

Hinz et al., (2012) revealed that the susceptibility of κ-casein to cross-linking increased with decreasing pH, whereas the susceptibility of β-casein to TGase-induced cross-linking was not affected. The order of susceptibility of the individual caseins in milk to TGase-induced cross-linked gradually changed from κ-casein >β-casein >αs1- casein at pH (5·5).

Moon et al. (2009) found that the increased susceptibility of κ-casein to TGase-induced cross-linking with pH decreasing, is probably due to the fact that a decrease in pH results in a reduced solvency of the κ-casein, due to a progressive loss of net-negative charge; this eventually leads to collapse of the κ-casein hairs on the micelle surface. So, the repulsion reduced, accessibility of κ-casein on the micelle surface is likely to be increased and the susceptibility of κ-casein to TGase-induced cross-linking thus also increased.

2.4.3. Enzyme Concentration.

Dinkci (2012) for strained yogurt, Aprodu et al. (2012) for non-fat set yogurt and Abdulqadr et al. (2014) for full-fat set yogurt, they all found that an increase in MTGase concentration led to an increase in their pH values as well as reduce their acidification rate. MTGase can reduce the growth of yogurt starters and their availability to the required nitrogen sources (amino acids and low-molecular-weight (LMW) peptides) through the formation of protein crosslinks, which can induce a longer lactic fermentation and a slower acidification processes (Lorenzen et al., 2002; Özer et al., 2007).

Abdulqadr et al., (2014); Li et al., (2015); Zhang et al., (2012) they all concluded that the increase of the MTGase concentration increases the protein crosslinking, which lead to an increase of the textural attributes.

Soleymanpuori et al., (2014) showed that increase in MTGase concentration provided a delay in the bacterial multiplication. It seems that the starter bacteria, especially Lb. delbrueckii subsp. bulgaricus at higher MTGase levels, have a lower accessibility to the specific nitrogen sites of the cross-linked proteins, leading to a reduction of their survival rate.

Concomitant increase of cottage cheese hardness with concentration increasing of the added TGase was reported by Cozzolino et al. (2003) and Han et al. (2003) who found that the texture of cottage cheese was improved upon MTGase treatment.

Cancino et al, (2006) they Showed that MTGase concentration affect WHC and syneresis properties, so that an increase in MTGase concentration is resulted in a decrease in syneresis rate and an improvement in WHC quantity.

Özer (2007) reported that Acid development rate was reduced with increasing MTGase doses, yogurt starter bacteria growth slowed, and the physical properties of the yogurts were improved during storage period (21 day). However, the production of acetaldehyde was slowed down by increasing MTGase concentrations during the same period (21 day).

Dinkci (2012) found that higher treatments of the TG enzyme (1.29 Unit TG g-1 protein) and (1.85 Unit TG g-1 protein) decreased the proteolytic activity and acidity with increasing storage time. On the contrary enzymatic cross linking had no significant effect on the microbiological properties and the sensory attributes were not unfavorable affected.

2.4.4. Presence of Enzyme Cofactors.

Activation of transglutaminase depends on the availability of metal ions (Sidauruk et al. 2017). The presence of divalent cations (i.e., Cu^{+2}, Zn^{+2}, Pb^{+2}, Li^{+2}, and Mg^{+2}) inhibits enzyme activity, and the activity and thermal stability of the enzyme are increased in the presence of monovalent ions, such as Na^{+} and K^{+} (Macedo 2009; Kütemeyer et al. 2005).

The purified TG was strongly inhibited by Pb^{+2}, $Zn^{+2}$and Cu^{+2}, and moderately inhibited by Ni^{+2}, $Co^{+2}$and Fe^{+2}. However, K^{+}, Na^{+}, Mg^{+2}, Ca^{+2} $Mn^{+2}$and Ba^{+2} did not affect the activity of the purified TG. Because heavy metals such as Pb^{+2}, Zn^{+2} and Cu^{+2} bind the thiol group of the single cysteine residue, this strongly supports the idea that a cysteine residue could be part of the active site of TG (Tsai et al. 1996; Motoki and Seguro 1998).

2.4.5. Time of Enzyme Addition.

Mahmood and Sebo (2009) investigate the effect of MTGase addition time on the physical and sensory properties of the soft cheese as a function of storage time. Results showed that The addition of the enzyme to the milk prior to rennet addition caused the prevention of milk coagulation. The second treatment included the addition of MTGase to the milk with the rennet at the same time. This treatment caused a significant drop in the curd and cheese strength accompanied by a noticeable loss of protein and fat in the whey. The third trial was carried out by adding the MTGase after coagulation and curd cutting, which was the most suitable way. This treatment was undertaken in order to avoid the inhibitory effect of MTGase against clotting enzyme activity. Results showed a significant drop in whey protein content and improvement in cheese hardness as a function of the added MTGase concentration.

Piero et al., (2010) found that TG may also be added into the cheese curd after cutting instead of being added to cheese milk. In this case, along with the increase in water content of the curd, the protein level of the curd may also increase. Similarly, De Sa and Bordignon-Luiz (2010) demonstrated that addition of TG after rennet resulted in an increase in consistency in processed cheeses. Incorporation of TG to milk 30 min after rennet addition led to a remarkable decrease in protein level of cheese whey (Cozzolino et al., 2003).

More recently, Yüksel, Avci, and Erdem (2011) showed that regarding the reaction kinetics, TG should be added to cheese milk 5 min before rennet addition.

Özer et al., (2013) found that simultaneous use of microbial transglutaminase (MTGase) and rennet in white- brined cheese production resulted in higher yield values for experimental cheeses than for the control cheeses, the total solids contents of the cheeses treated with MTGase were remarkably lower than the control cheeses; but the former cheeses had higher protein-in-dry matter levels.

2.4.6. Milk Heat Treatment.

2.4.6.1. Influence of Heat Treatment on Different Dairy Products.

Heat treatment is the most widely used processing technology in the dairy industry. Its main purpose is to destroy microorganisms, both pathogenic and spoilage, to ensure the milk is safe and has a reasonable shelf life. furthermore, heat treatment affects the flavor, viscosity and milk proteins. Different studies investigate the effect of pasteurization temperature on the physical properties of different dairy products.

Marshall et al. (1978) found that the increased yield (not given) of Cheddar cheese manufactured with replacement of one-third or one-half of the pasteurized milk (72°C/15 s) with milk heated at (87.8°C/17 s) was due entirely to greater moisture retention.

Heat treatment greater than pasteurization (i.e. 72°C/16 s) has been used to increase yields of Cottage cheese and acid set Mozzarella (Kannan and Jenness 1956, Buchanan et al. 1965).

Singh and Waungana (2001) concluded that, when heating cheese milk above (65 C∘), the whey proteins denatured by the unfolding of their polypeptides, thus exposing the side chain groups originally buried within the native structure. The unfolded proteins then, interact with casein micelles or simply aggregate with themselves. because of this, the effect of high temperature pasteurization on whey proteins is desirable since heat treatment of the milk cause whey proteins incorporation into cheese curd, resulting in a higher yield from a given quantity of milk.

Meinardi. et al., (2003) reported that the pasteurization temperature had strong positive effect on cheese yield. Cheeses made from pasteurized milk to (75 C°) had higher moisture content, that due to the presence of whey proteins, since their hydrophilic property allowed higher moisture retention.

Rynne et al., (2004) investigate the influence of different pasteurization temperatures in whey protein denaturation. Half-fat Cheddar cheeses (~15%, w/w, fat) were manufactured from milk pasteurized at (72 C°, 77 C°, 82 C° or 87 C°) for (26 s), and analyzed over a (360) days ripening period. The mean levels of whey protein denaturation in the pasteurized milks were (2.8%, 8.4%, 20.2% and 34.1%) of total whey protein, respectively. The study concluded that, increasing pasteurization temperature will significantly increase the levels of moisture, and decreased the levels of protein, fat, calcium in cheddar cheese.

Şimşek, and Sagdic (2012) showed that the yield and some physicochemical properties, which are including total solids and pH, of the Çökelek cheese samples heated at (95°C) were better than those of the samples heated at (85°C). However, the sensorial score, ripening index and lactic acid bacteria counts of the samples heated at (85°C) were higher than those heated at (95C°).

Faru. et al. (2014) indicated that, goat milk pasteurization at (75 C∘) for (30) minutes has a positive impact on spreadable cheese yield, and sensory properties. The average cheese yield for samples elaborated from milk pasteurized at (75 C∘) and using autochthonous goat culture was (32.4%) (3.24 L milk/Kg cheese).

2.4.6.2. Influence of Heat Treatment on TG-reactions.

Caseins, the principle proteins in milk, are particularly good substrates for TG. This is probably due to their low degree of tertiary structure, flexible, random-coil arrangement and the absence of any disulphide bonds, leaving the reactive groups exposed to the enzyme (Faergemand et al. 1999; O'Connell and De Kruif, 2003). However, whey proteins tend to cross-link less efficiently; Due to their compact globular structures (Sharma et al. 2002).

The globular whey proteins are hardly susceptible to TG enzyme. Therefore, it requires steps that promote their partial denaturation to make them accessible to enzyme activity (DeJong and Koppelman, 2002).

During heat treatment of milk, whey proteins interact with casein micelles (Han & Damodaran 1996) and the main complex formed is the β -lactoglobulin/k-casein complex (Hill, 1989; Jang & Swaisgood, 1990).

Although **α** -lactalbumin cannot associate with the micelle in the absence of β -lactoglobulin, it forms a heat-induced β -lactoglobulin/α-lactalbumin complex, which then interacts with k-casein (Elfagm & Wheelock, 1978). These reactions appear to enhance the susceptibility of milk proteins to transglutaminase reactions by means of a closer approach of the proteins at the active site of the enzyme transglutaminase and the subsequent formation of polymers (Sharma et al., 2001).

Aboumahmoud and Savello (1990), and Sharma et al., (2001) found that, milk proteins in unheated skim milk were susceptible to TG crosslinking, while milk preheating at (85 C°/ 15 min) enhances milk protein susceptibility toward TG crosslinking.

Han and Damodaran (1996) showed that the modification of the whey proteins by preheating of the milk before incubation with transglutaminase may have allowed formation of hetero-polymers between the caseins and whey proteins.

Lauber et al., (2001) have demonstrated that globular proteins like whey proteins can be modified by transglutaminase after denaturation under high-pressure treatment.

Bönisch et al., (2004) concluded that, ultra-high temperature (UHT) treatment results in higher amount of TG crosslinks.

Rodriguez-Nogales, (2006) concluded that milk proteins were susceptible to transglutaminase mediated cross-linking in unheated and heated milks, however, the transglutaminase action was more efficient in heated samples of milk. k-casein and β- caseins are more susceptible to a transglutaminase reaction than the αs1-casein and αs2-casein when a preheating treatment was employed. This behavior is due to the open structure of the casein micelle, where α-casein forms the backbone structure whereas β -casein is in a dynamic state with a disordered, flexible and open structure that facilitates the crosslinking (Sharma et al. 2001).

Mazuknaite et al., (2013) concluded that heating transglutaminase-enhanced skim milk to (40C°) or (50C°) for (20–60) min increases the rate of protein polymerization and cheese yield.

2.5. Influence of Transglutaminase Cross-linkage on Fermentation Time and Rennet Clotting Time.

Lorenzen et al., (2002), found no reduction in fermentation time when using MTGase but concluded that the addition of MTGase led to greater viscousness and protein polymerization when compared with the control (without enzyme) (Bönisch et al., 2007a, b).

Lorenzen et al., (2002); and Ozer et al., (2007) they reported that MTGase can be applied either to the lactic acid culture or prior to fermentation. When applied prior to fermentation, the fermentation time takes longer because the peptides available (filled by cross-linking) for microbial growth are reduced, although this process offers the advantage of constant pH during the formation of cross-links. In this case, after incubation, a heat treatment is applied to deactivate the enzyme.

Benković et al. (2008) had previously indicated that the fermentation time of full-fat probiotic yogurts containing inulin and MTGase is shorter than the control ones. They suggested that the acceleration of the fermentation process is related to the affinity of the probiotic strain Bifidobacterium animalis subsp. lactis LAFTI® B94 to hydrolyze the inulin prebiotic carbohydrate.

Abd Rabo et al., (2014); Romeih et al., (2014); and Tsevdou et al., (2013) showed that the incorporation of MTGase had no remarkable impact on the fermentation time in non-fat, full-fat set yogurts, and also non-fat stirred types as showed by Bönisch et al., (2007a); and Bönisch et al, (2007b).

Abdulqadr et al. (2014) showed that the fermentation time of full-fat set yogurt can be extended from (180 to 210) min by increasing the MTGase content in the range of (2–8) U/g. L. delbrueckii subsp. bulgaricus is known to have proteolytic activities, whereas the S. thermophilus represents either weak or no proteolytic activities. Thus, the proteolytic lactobacilli by liberating amino acids provide the most important growth-promoting sources for S. thermophilus. In addition, other growth stimulating parameters such as formic acid and CO_2 can be provided by S. thermophilus for the lactobacilli (Tamime & Robinson, 1999). MTGase by forming protein crosslinking between LMW peptides and/or amino acids, probably delays the release of amino acids required for the proteolytic activity of L. delbrueckii subsp. bulgaricus, leading to an increase of the required incubation time.

One major problem with TG application in cheese manufacturing is that the conditions optimum for TG activity may well hinder the activity of rennet (Bönisch et al.,2008).

TG is expected to slow down the release of caseinomacropeptide (CMP) during the primary phase of coagulation, a mechanism triggered by chymosin (Bönisch et al., 2008). In this sense, application of TG and chymosin is expected by competing reactions (Özer et al., 2013).

Lorenzen (2000b) demonstrated that the surface of casein micelle was partly covered by whey proteins (mainly β-lactoglobulin) via heat-induced interactions with κ-casein, and this sterically slowed down the release of CMP from the micelle surface and extended coagulation time when TG was used prior to rennet addition. In addition, protein cross-linking induced by microbial transglutaminase (MTGase) would also prolong the rennet coagulation time of milk (Huppertz & de Kruif, 2007).

Lorenzen (2000b) and O'Sullivan et al. (2002) observed that TGase treatment increased the rennet coagulation time considerably with an increasing degree of cross-linking.

Lorenzen (2000b); Lorenzen & Schlimme, (1998) reported that the renneting ability loss of TGase treated milk could be explained by the 'surface sealing' of casein micelles with cross-linked whey proteins, especially β-lactoglobulin. This phenomenon may prevent cleavage of κ-casein. The cross-linking effect may be comparable with binding of denaturated β-lactoglobulin to κ-casein during heat treatment.

O'Sullivan et al. (2002) concluded that the TGase-induced inhibition of the rennet-induced coagulation of milk was largely due to inhibition of the primary stage of rennet coagulation. Huppertz and de Kruif (2007) reported that this phenomenon resulted primarily from an inhibition of the secondary phase of rennet coagulation. Bönisch et al. (2008) reported that enzymatic cross-linking affects both the primary and the secondary stage of rennet coagulation.

Domagała et al. (2016) found that the coagulation time was affected by transglutaminase and gel texture. The longest rennet coagulation time (>91% vs. control) and the lowest gel firmness (<38% vs. control) was observed at the highest concentration of transglutaminase (3 U/g) with incubation at (40C°) for (2) h.

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AL- Quds University

CHAPTER THREE

MATERIALS AND METHODS

Chapter Three Materials & Methods

3.1.Introduction

This chapter present the research investigations, implementation mechanisms, and changes on the products during the storage period. In this section, the sources of materials used in the manufacture of concentrated yogurt (Labneh) and white- brined cheese (WBC), experimental design (including the methods of manufacturing Labneh and local white- brined cheese, yield percentage determination – for both products-, in addition to whey percentage determination - only for Labneh-, and sampling), physicochemical, and microbial analysis during the storage period, in addition to statistical analysis will be exhibited. All analytical procedures were carried out according to Bacteriological analytical manual (BAM), or published articles.

3.2. Materials

3.2.1. Milk

Unpasteurized fresh milk of morning milking was purchased from a local market in Abo-dis area, Jerusalem. The chemical composition of the raw milk outlined in table (3.1), which represent the raw milk composition for both products (concentrated yogurt, and local white- brined cheese). The chemical composition of the raw milk was determined using milk analyzer (Lactoscan S standard model). The analysis was carried out four times for each product raw milk.

Table (3.1): The chemical composition of raw milk.

Milk Component	Concentrated yogurt raw milk composition (Average% ±SD)	Local white- brined cheese raw milk composition (Average % ±SD)
Fat	4.21± 1.35	4.49± 1.79
Lactose	4.17± 0.16	4.11± 0.22
Protein	2.94± 0.1	2.91± 0.14
pH	6.94±0.19	6.89± 0.05

3.2.2. Transglutaminase Enzyme (TG)

It was purchased from Omega raw materials and drug store company, Nablus. Enzyme potency was (115-155 U/g powder).

3.2.3. Source of Salt and Traditional Yogurt

For concentrated yogurt (Labneh) production, clean iodized salt (NaCl) in a finely powdered form, and traditional yogurt produced by local company were purchased from a local market.

3.2.4. Calcium Chloride and Rennet Enzyme

For white- brined cheese production, calcium chloride ($CaCl_2$) (Herbstreith and Fox), and rennet enzyme (Valiren® Granular Thermostable, with standard activity of (2250 IMCU/g) that used from the Food processing laboratories, Food engineering department, Al-Quds university.

3.3. Methods

Traditional manufacturing methods were used, with modifications according to the objectives of the study, that will be presented in the next sections.

3.3.1. Manufacture of Concentrated Yogurt (Labneh).

Labneh experiments were applied at Al-Quds University food processing laboratories. Four heat treatments (HT1- pasteurization (65C°/ 30min), HT2 – pasteurization (72C°/ 15 sec), HT3 – pasteurization (82C°/ 15 sec), and HT4 – pasteurization (90C°/15 sec)) were applied to the raw milk. For each heat treatment, milk cooled to (42C°). The treated milk then was divided equally into (4) parts, according to the experimental design outlined in table (3.2). The first part (control), with no TG enzyme addition, only (3%) of the traditional culture added. The second part, TG enzyme were added by the ratio (1gm TG/kg milk) simultaneously (Darnay et al., 2016) with (3%) traditional culture. The third part, TG enzyme added by the ratio of (3gm TG/kg milk) simultaneously with (3%) traditional culture. The fourth part, TG enzyme was added by the ratio of (5gm TG/kg milk) simultaneously with (3%) traditional culture. The mixtures then mixed to distribute TG enzyme and the traditional culture through the milk mass. The mixtures were incubated at (42C°) for (6) hrs. until complete coagulation. The resultants coagulated mass was mixed thoroughly with (1.5%) NaCl and then transferred to clean cheesecloth bags, hanged to drain the whey in the refrigerator for about (22) hrs. The obtained Labneh then, packaged in polyethylene bags, and kept in refrigeration temperature for further analysis.

Table (3.2): Experimental milk design for Labneh production with TG enzyme.

Heat treatment (HT)	Experimental part		Traditional Starter culture	TG enzyme ratio
HT1	First (control)	Z	3%	0
	Second	A	3%	1gm TG/kg milk
	Third	B	3%	3gm TG/kg milk
	Fourth	C	3%	5gm TG/kg milk
HT2	First (control)	Z	3%	0
	Second	A	3%	1gm TG/kg milk
	Third	B	3%	3gm TG/kg milk
	Fourth	C	3%	5gm TG/kg milk
HT3	First (control)	Z	3%	0
	Second	A	3%	1gm TG/kg milk
	Third	B	3%	3gm TG/kg milk
	Fourth	C	3%	5gm TG/kg milk
HT4	First (control)	Z	3%	0
	Second	A	3%	1gm TG/kg milk
	Third	B	3%	3gm TG/kg milk
	Fourth	C	3%	5gm TG/kg milk
HT1- pasteurization 65C°/ 30min, HT2 – pasteurization 72C°/ 15 sec, HT3 – pasteurization 82C°/ 15 sec, and HT4 – pasteurization 90C°/15 sec				

3.3.2. Yield and Whey Percentages Measurements.

In order to determine the optimum combination of pasteurization temperature and TG ratio that give the highest yield and lowest whey loss, yield and whey percentage of fresh Labneh samples were calculated.

Yield and whey values were calculated according to Sumarmono et.al (2019). Data obtained were expressed as a percentage.

3.3.3. Concentrated Yogurt (Labneh) Making Flow Chart.

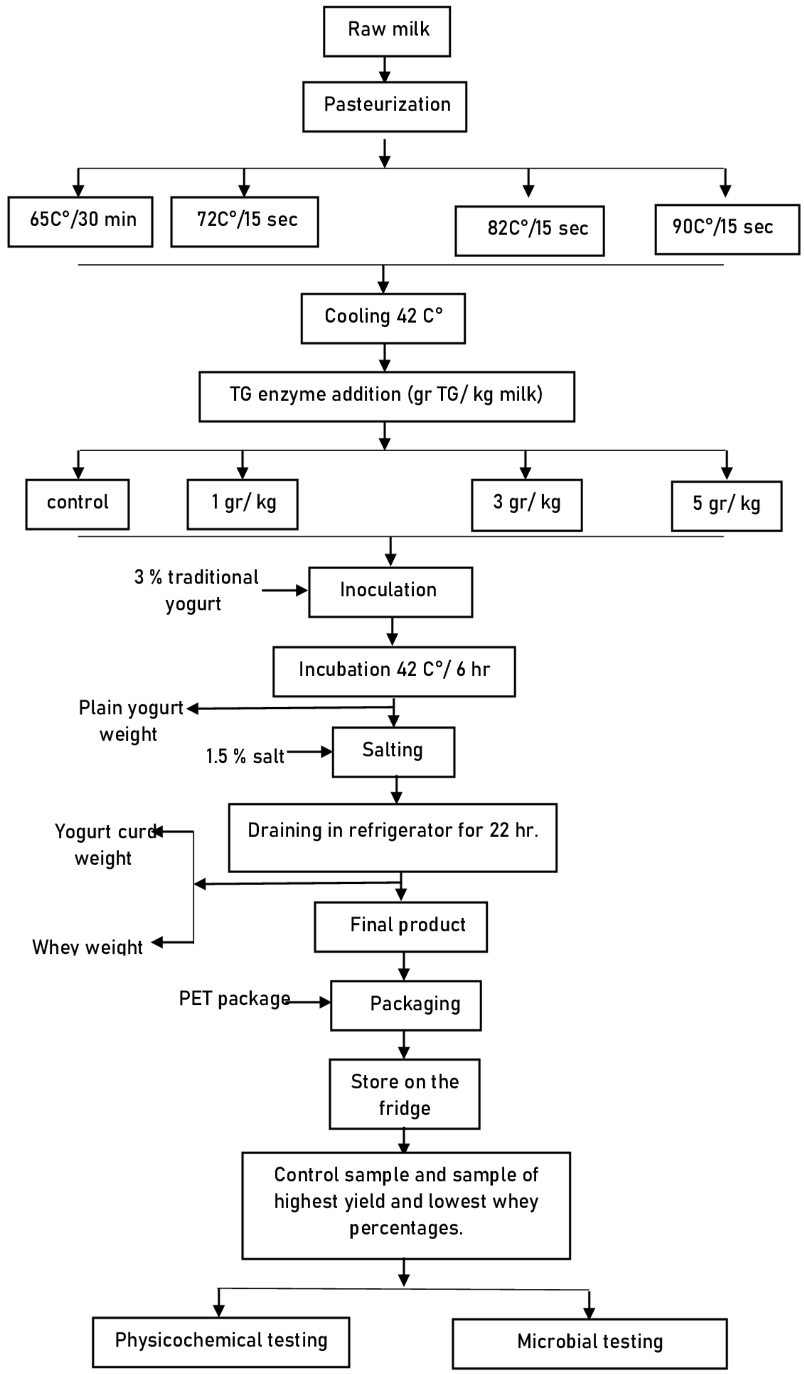


Fig.3.1: Representative flow chart of the concentrated yogurt (Labneh) making.

3.3.4. Manufacture of Local White- Brined Cheese (WBC)

Experimental cheeses were produced at Al-Quds University food processing laboratories. Three heat treatments (HT1- pasteurization (65C°/ 30min), HT2 – pasteurization (72C°/ 15 sec), and HT3 – pasteurization (82C°/ 15 sec)) were applied to the raw milk. For each heat treatment, milk was cooled to (32C°), then (0.01%) of calcium chloride ($CaCl_2$) added to the milk, and holding milk at (35C°) for (30 min). The milk was divided into four batches, according to the table (3.3), which outlined the experimental batches. The first batch (control), (0.03%) of rennet enzyme added, TG enzyme not added. The second batch, TG enzyme added at a ratio of (1gm/kg milk), simultaneously with rennet enzyme (Özer et al., 2013). The third batch, TG enzyme added at a ratio of (3gm/kg milk), simultaneously with rennet enzyme. The fourth batch, TG enzyme added at a ratio of (5gm/kg milk), simultaneously with rennet enzyme. All batches then, incubated at (32 C°) for (45 min). After which, the resultant coagulum was cut into (1 cm^3) pieces and left for (20 min). The curd pieces were transferred to clean cheesecloth bags for whey removal by pressing in the refrigerator for (12 hr.). The fresh cheeses were portioned and kept in glass jars containing pasteurized brine solution (14% NaCl). The cheeses were stored in refrigeration temperature.

Table (3.3): Experimental milk design for local WBC production with TG enzyme.

Heat treatment (HT)	Experimental batch		TG enzyme ratio	Rennet enzyme
HT1	First (control)	Z	0	0.03%
	Second	A	1gm TG/kg milk	0.03%
	Third	B	3gm TG/kg milk	0.03%
	Fourth	C	5gm TG/kg milk	0.03%

HT2	First (control)	Z	0	0.03%
	Second	A	1gm TG/kg milk	0.03%
	Third	B	3gm TG/kg milk	0.03%
	Fourth	C	5gm TG/kg milk	0.03%
HT3	First (control)	Z	0	0.03%
	Second	A	1gm TG/kg milk	0.03%
	Third	B	3gm TG/kg milk	0.03%
	Fourth	C	5gm TG/kg milk	0.03%
HT1- pasteurization 65C°/ 30min, HT2 – pasteurization 72C°/ 15 sec, and HT3 – pasteurization 82C°/ 15 sec.				

3.3.5. Yield Measurement

In order to determine the optimum combination of pasteurization temperature and TG ratio that give the highest yield, yield percentage of fresh cheese samples were calculated.

Yield was calculated according to Spadoti et al. (2003). Data obtained were expressed as a percentage.

3.3.6. Local White- Brined Cheese (WBC) Making Flow Chart.

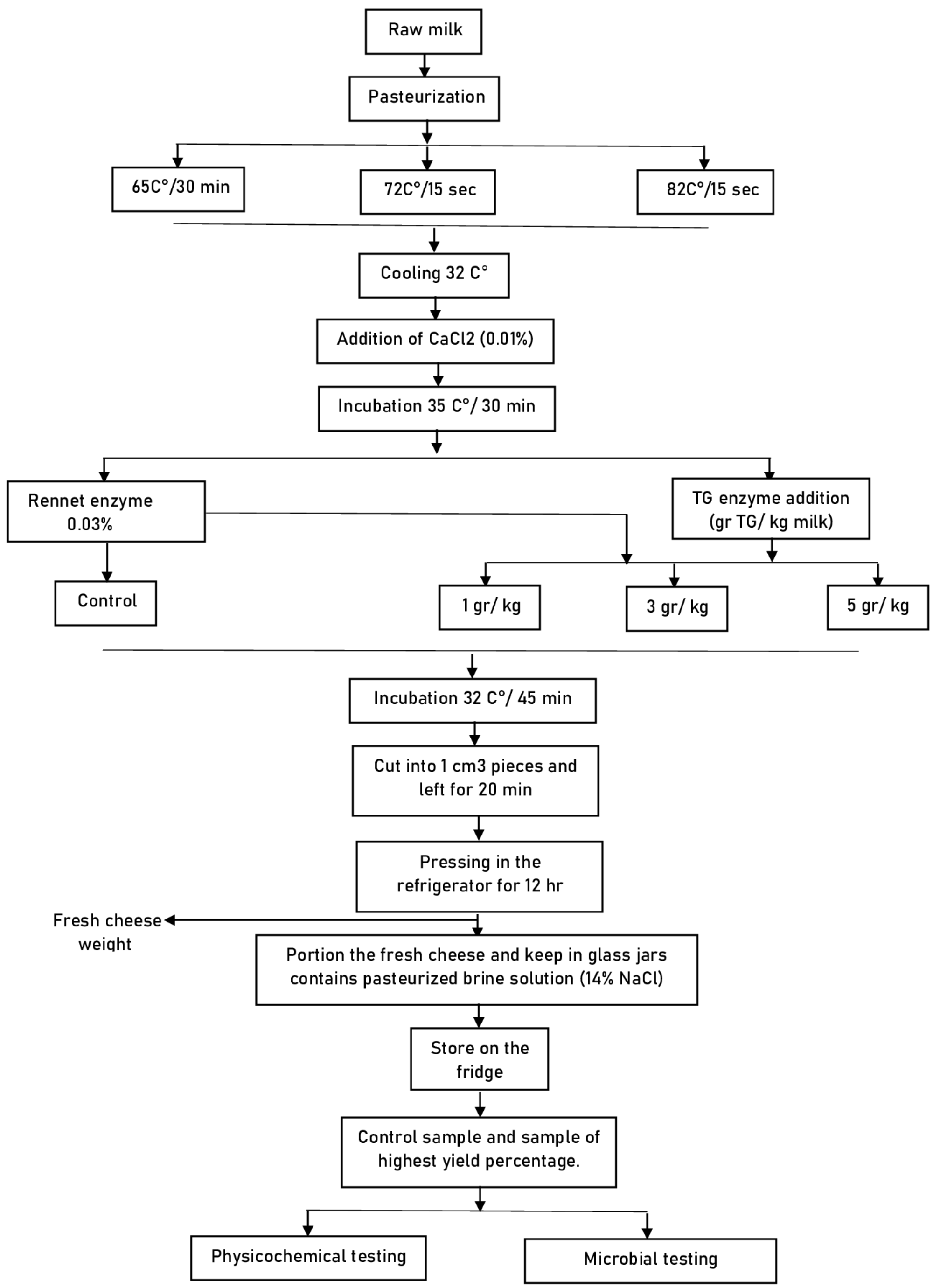


Fig.3.2: Representative flow chart of the local white brined cheese (WBC) making.

3.3.7. Sampling

According to the highest yield results, control and treated samples of Labneh and white-brined cheese weighing (500-700) grams were stored in refrigeration. At different periods of storage time (0 day, 15 days, and 30 day), the enzyme-treated samples and the control samples were evaluated from a physicochemical, and microbial aspects. Each test was carried out in triplicate.

3.4. Physicochemical Analysis

3.4.1. Syneresis, and WHC of Concentrated Yogurt (Labneh)

Syneresis and water holding capacity (WHC) of Labneh samples were calculated using centrifuge device (Hettich type) according to Sumarmono et.al (2019). Data obtained were expressed as a percentage.

3.4.2. Moisture and Dry matter (DM) Contents of White- Brined Cheese

Moisture and dry matter contents of the cheese samples were determined using a digital moisture analyzer (Sartorius type) according to Frau et al. (2014). The obtained results were expressed as moisture percentage.

3.4.3. Titratable Acidity

Titratable acidity of Labneh and white- brined cheese samples were determined according to the method expressed by Bradley et al (1992). The titratable acidity results were presented as lactic acid%.

3.4.4. pH

For Labneh and white- brined cheese, pH value was measured using a digital pH meter (HACH type- HQ 11d), after calibration using fresh standard buffer solutions at (4.0 and 7.0) of pH (Ardö, Y., & Polychroniadou, 1998).

3.5. Microbiological Analysis

3.5.1. Lactic Acid Bacteria (LAB)

Lactic acid bacteria count of Labneh samples was measured based on the double layer pour plate approach using MRS agar medium (HIMEDIA). (Abdalla & Omer, 2017; Mucchetti et al., 2008).

3.5.2. Yeast and Mold

Yeast and molds colonies of Labneh and white- brined cheese samples were determined by pour plate technique using potato dextrose agar (HIMEDIA). (Harrigan, 1998).

3.5.3. Total Bacterial Count (TBC)

The total bacterial viability of white- brined cheese samples was measured based on the pour plate techniques using the Plate count agar (HIMEDIA) culture media (Eljagmani and Altuner, 2020).

3.5.4. Total Coliform (TC)

Violet red bile agar (HIMEDIA) was used for the enumeration of coliforms in white- brined cheese samples. (Abdalla & Omer, 2017; Alper & Nesrin, 2013; Mucchetti et al., 2008).

3.6. Statistical Analysis

Statistical analysis of the three replicates of each sample was determined by analysis of variance (ANOVA, Fisher LSD, $p < 0.05$) using XLSTAT software version 7.5.2 (Addinsoft, USA).

The experimental procedures and techniques are detailed in appendix one and two.

AL- Quds University

CHAPTER FOUR

RESULTS AND DESCUSIONS

Chapter Four Results and Discussions

4.1. Concentrated Yogurt (Labneh).

4.1.1. Influence of Different Pasteurization Temperatures, combined with Different Milk TG Treatments on the Concentrated Yogurt Yield and Whey Percentages.

The obtained results of each pasteurization temperature will be discussed separately according to the added TG enzyme concentrations, and presented in figures for better illustration.

4.1.1.1. Influence of (65C°/ 30 min) Pasteurization Temperature combined with Different Milk TG Treatments on the Concentrated Yogurt Yield and Whey Percentages.

At (65C°/ 30 min) pasteurization temperature, yield and whey of the concentrated yogurt (Labneh) samples were calculated as a percentage.

The results (Fig.4.1) showed that the yield percentage significantly increased when TG enzyme added, compared to the control sample. The control sample (Z) yield value was (25.53±1.5), whereas, the yield values of TG treated samples with ratios of (1gm TG/Kg milk, 3 gm TG/Kg milk, 5gm TG/Kg milk) were (28.98±0.2, 29.91±0.49, and 29.32±1.01, respectively). However, there was no significant difference between the yield percentages at different TG enzyme concentrations.

Regarding to whey percentage, the obtained results indicated that, control sample (Z) exhibited significantly higher whey percentage (Fig.4.1). On the other hand, there was a little significant difference between the whey percentage obtained from the control sample (Z) and that obtained from the concentrated yogurt sample (A) with (1gm TG/Kg milk) enzyme ratio, the whey percentages were (71.42±2.98, and 69.86±2.54, respectively).

In overall, the percentage of whey separated from the concentrated yogurt samples decreased as the TG enzyme concentration increased.

From the previously mentioned results (Fig.4.1), it can be revealed that the TG ratios that gave the highest yield and lowest whey loss at (65C°/ 30min) pasteurization temperature were (3 gm TG/Kg milk, and 5gm TG/Kg milk).

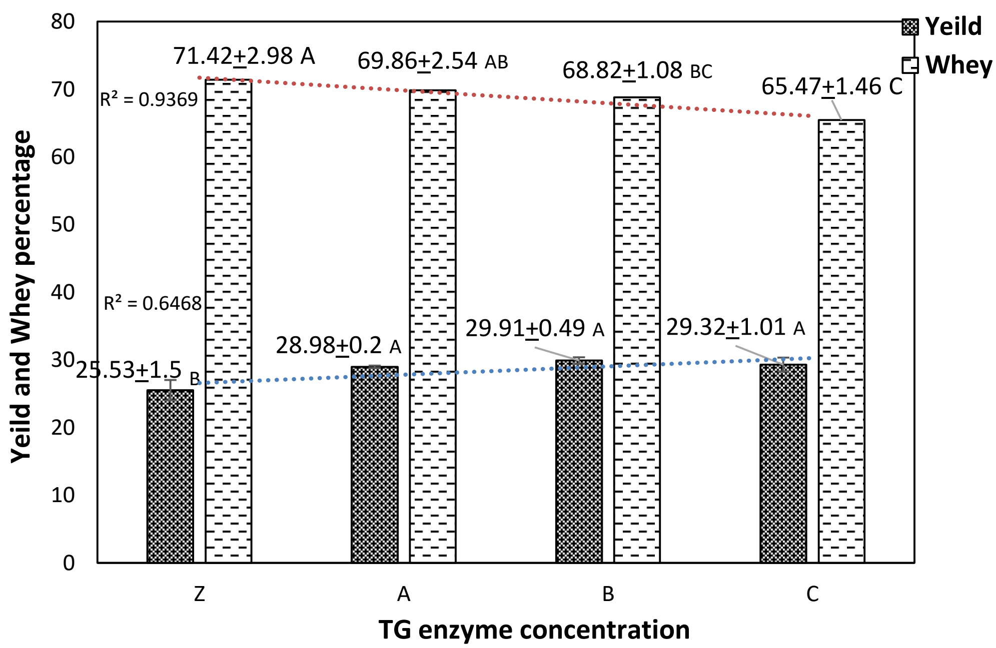


Fig.4.1: Influence of (65C°/ 30 min) pasteurization temperature combined with different milk TG treatments on the concentrated yogurt yield and whey percentages. ± Letters (A - D) indicate the statistical differences (at 0.05 level, Fisher test) between different

4.1.1.2. Influence of (72C°/ 15) Pasteurization Temperature combined with Different Milk TG Treatments on the Concentrated Yogurt Yield and Whey Percentages.

When (72C°/ 15) pasteurization temperature applied, the obtained results showed a significant difference between the control and TG samples regarding yield and whey percentages (Fig.4.2).

The significant higher yield percentage was obtained when (3gm TG/ kg milk) ratio applied (B sample), the yield value was (26.24±1.29). The yield percentage when (5 gm TG/kg milk) ratio applied (C sample) decreased significantly to value relatively similar to that obtained when (1gm TG/ kg milk) ratio applied (A sample), the yield values were (24.81±0.24, and 23.62±0.02, respectively).

Significant difference obtained on the whey percentages when (72C°/ 15) pasteurization temperature applied with different TG concentrations (Fig.4.2).

The significant higher whey percentage found on the control (Z sample) with a value of (76.27±0.17), followed by A sample at (1 gm TG/ kg milk) with a whey percentage of (73.57±0.69), followed by C sample with a value of (72.17±0.38), and the lowest value showed by B sample at (3 gm TG/ kg milk) which was (68.61+1.06).

By comparing the previously mentioned results, the best TG enzyme ratio that gave the highest yield and lowest whey loss, at (72 C°/ 15) pasteurization temperature was (3 gm TG/ kg milk).

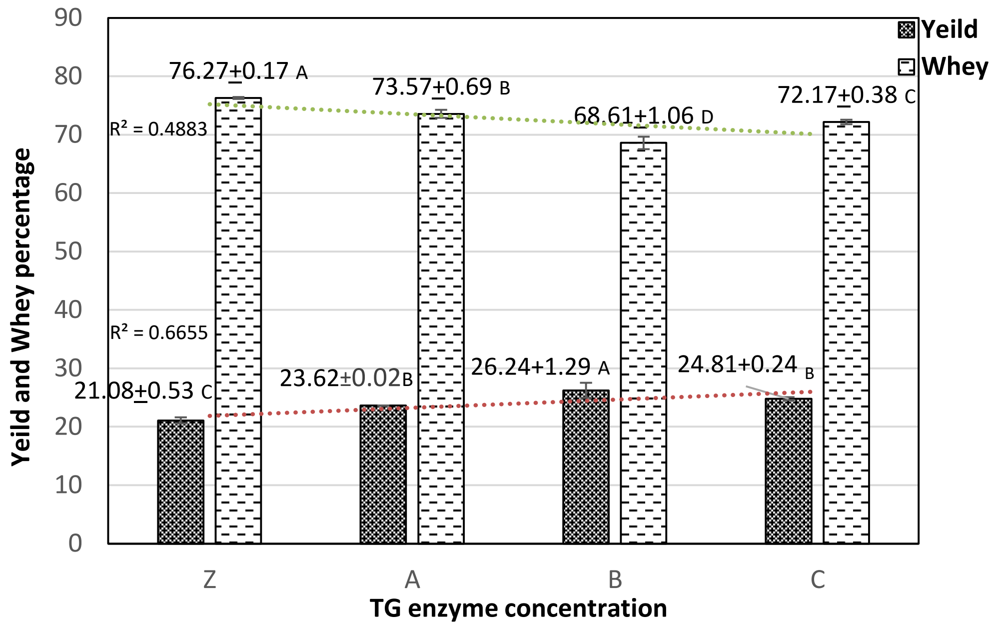


Fig.4.2: Influence of (72 C°/ 15 sec) pasteurization temperature combined with different milk TG treatments on the concentrated yogurt yield and whey percentages. ± Letters (A - D) indicate the statistical differences (at 0.05 level, Fisher test) between different TG treated samples.

4.1.1.3. Influence of (82C°/ 15) Pasteurization Temperature combined with Different Milk TG Treatments on the Concentrated Yogurt Yield and Whey Percentages.

Yield percentage results significantly increased when TG enzyme ratio increased as shown in Fig (4.3).

The control sample (Z sample) represented the lower yield value (22.93±0.43). The yield percentages significantly increased as the TG enzyme ratio increased. C sample exhibited the significant higher yield value (29.63±0.16), followed by B sample (29.12±0.58). The lower yield percentage obtained when (1 gm TG/ kg milk) ratio applied (A sample). A small significant difference showed between the TG samples yield results as the enzyme ratio increased.

On contrary, the whey percentage results decreased significantly with TG enzyme addition (Fig.4.3).

The whey percentage result of the control sample (Z sample) was (73.74±0.61). A significant decrease observed in the whey percentage result of A sample (69.86±1.24), the decrease continued as it exhibited by B sample whey percentage result (65.61±1.29). There is no significant difference between the whey percentage of B sample and C sample (65.61±1.29 65.89±0.06, respectively).

Concluding the previously mentioned results, the TG enzyme ratios that gave higher yield and lower whey loss at (82 C°/ 15) pasteurization temperature were (3 gm TG/ kg milk, and 5 gm TG/ kg milk).

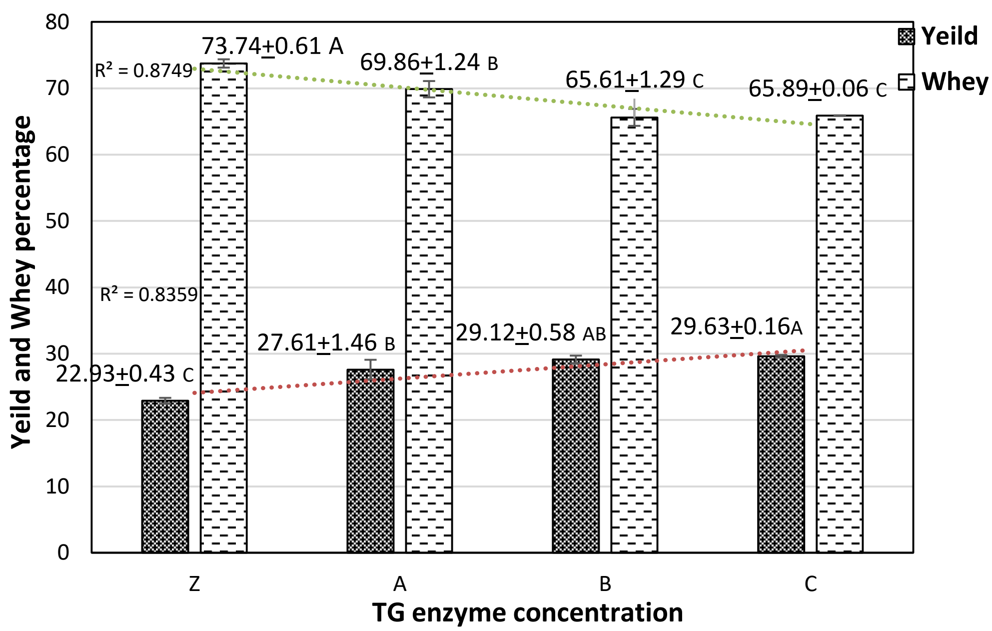


Fig.4.3: Influence of (82 C°/ 15 sec) pasteurization temperature combined with different milk TG treatments on the concentrated yogurt yield and whey percentages. ± Letters (A - D) indicate the statistical differences (at 0.05 level, Fisher test) between different TG treated samples.

4.1.1.4. Influence of (90C°/ 15) Pasteurization Temperature combined with Different Milk TG Treatments on the Concentrated Yogurt Yield and Whey Percentages.

According to Fig. (4.4), the yield percentage significantly increased when TG enzyme applied respect to the control sample (Z sample). These findings showed that, control sample result was (29.83±0.91). On the other hand, the yield percentage results of TG samples were as the following: (31.88±0.82) for sample A (1 gm TG/ kg milk), (31.89±0.84) for B sample (3 gm TG/ kg milk), and (31.22±0.63) for C sample (5 gm TG/ kg milk). There is no significant difference between A sample and B sample. However, a small significant difference was observed between C sample and the other TG samples.

Compared to the control sample (Z sample), the whey percentage decreased when TG enzyme applied (Fig.4.4).

There is a little significant difference between the whey percentage results of the control sample (67.16±0.89), A sample (64.7±1.14), and C sample (64.5±0.61). No significant difference showed between A and C samples. However, a significant difference was observed between B sample (3 gr TG/ kg milk) and the control sample (Z sample), the whey percentage results were (61.68±4.38, and 67.16±0.89, respectively). Comparing B sample to the others TG samples, a small significant difference exhibited. Concluding the obtained results above, the optimum TG ratio used when (90 C°/ 15) pasteurization temperature applied were (1 gm TG/ kg milk), and (3 gm TG/ kg milk), as the higher yield results were found. While in terms of whey percentage, TG enzyme ratio of (3 gm TG/ kg milk) gave lower whey loss than (1 gm TG/ kg milk) ratio. Which summarize that the best ratio that gave the higher yield and lower whey loss at (90 C°/ 15 sec) pasteurization temperature was (3 gm TG/ kg milk).

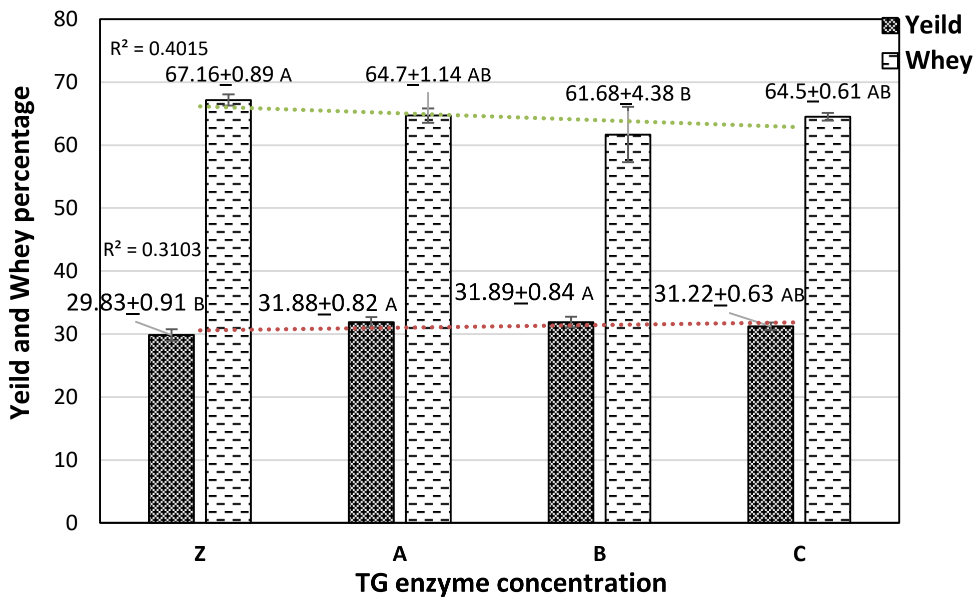


Fig.4.4: Influence of (90 C°/ 15 sec) pasteurization temperature combined with different milk TG treatments on the concentrated yogurt yield and whey percentages. ± Letters (A - D) indicate the statistical differences (at 0.05 level, Fisher test) between different TG treated samples.

As a summary, the previously mentioned results at the different pasteurization temperatures showed that the TG enzyme addition cause a significant change in the yield and whey percentages of the concentrated yogurt samples.

The increase in yield percentage and decrease in whey percentage with TG enzyme addition can be explained by the enzyme action, which stimulate the formation of new intra- and inter-molecular crosslinks between the milk proteins, the additional crosslinks increase the gel strength, reduce whey loss, and increase water holding capacity (WHC) (Oner et al., 2008).

These findings were agreed with Gauche et al., (2009) who observed that crosslinking caused by TG enzyme has a direct influence on the rate of syneresis. Motoki and Seguro (1998) concluded that WHC of yogurt gel network improved by MTGase. Lauber et al., (2000); Faergemand et al., (1999) found that the TG enzyme triggered crosslinks between milk proteins, leading to decrease in gel permeability. This decrease causes more compact and stable microstructure with smaller compartments in yogurt. Hence, more free water entrapped in yogurt gel network, which reduce whey loss (Moon and Hong, 2003).

4.1.2. Comparison between the Highest Yield Samples at the Different Pasteurization Temperatures.

The concentrated yogurt samples that gave the highest yield percentages at the different pasteurization temperatures with the best TG ratio were showed in table (4.1).

Table 4.1: The highest yield concentrated yogurt samples at the different pasteurization temperatures with optimum TG ratio. ± Letters (A - D) indicate the statistical differences (at 0.05 level, Fisher test) between different TG treated samples.

Pasteurization temperature	Sample with highest yield %	Sample TG ratio	Yield %
65 C°/ 30 min	B	3 gm TG/ kg milk	29.91±0.49 A
72 C°/ 15 sec	B	3 gm TG/ kg milk	26.24±1.29 A
82 C°/ 15 sec	B	3 gm TG/ kg milk	29.12±0.58 AB
90 C°/ 15 sec	B	3 gm TG/ kg milk	31.89±0.84 A

By comparing the yield percentages of B samples, it appears that there an effect of the pasteurization temperature on the yield values of TG concentrated yogurt.

The highest yield obtained when (90 C°/ 15) pasteurization temperature was applied, followed by (65 C°/ 30 min) pasteurization temperature, then (82 C°/ 15) pasteurization temperature, and the lowest value obtained when (72 C°/ 15) pasteurization temperature was applied.

The highest yield that obtained when the highest pasteurization temperature (90 C°/ 15) was applied can be explained by:

1. The influence of heat treatment (HT) on milk proteins.

When the milk is heated above (65 C°), the whey proteins are denatured by unfolding their polypeptides. The unfolded proteins then interact with casein micelles or simply aggregate with themselves (Sigh and Waungana, 2001). The presence of whey proteins allowed higher moisture retention, due to their hydrophilic property, and thus higher yield obtained (Meinardi. et al., 2003).

These findings agreed with Şimşek and Sagdic (2012) who found that the yield and some physicochemical properties of Çökelek cheese samples heated at (95 C°) were better than those samples heated at (85 C°). Rynne et al. (2004) found that increasing pasteurization temperature of milk from (72 C°, 77 C°, 82 C°, and 87 C° for 26 secs), the whey protein denaturation increased (2.8%, 8.4%, 20.2%, and 34.1%, respectively) and thus moisture level increased significantly.

2. The influence of heat treatment on TG- induced reactions.

TG crosslinking of milk proteins is determined by the molecular structure and accessibility of glutamine and lysine residues (Sharma et al., 2001).

Casein proteins can be easily cross linked by TG enzyme, due to the high flexibility, absence of disulfide bonds, along with little or no secondary protein structure (Sharma et al., 2001). In comparison to the whey proteins with globular dense structure stabilized by disulfide bridges (Sharma et al., 2002). Therefore, globular whey protein requires steps that promote their partial denaturation to make them accessible to the enzymatic activity (DeJong and Koppelman, 2002; Bonisch et al., 2007).

This explanation was in accordance with Aboumahmoud and Savello (1990); Sharma et al. (2001); who found that milk proteins in unheated skim milk were susceptible to TG crosslinking, while milk preheating at (85 C°/ 15 min) enhance milk proteins susceptibility toward TG crosslinking. Bönisch et al. (2004) found that ultra-high temperature (UHT) treatment results in higher amount of TG crosslinking.

Based on the discussed explanation mentioned above, milk proteins were more susceptible to TG crosslinking when milk undergo heat treatment. As the HT affects the protein structure and causes unfolding that led to protein interactions. These reactions appear to enhance susceptibility of milk proteins to TG reactions (Sharma et al., 2001).

As a conclusion based on the previously mentioned explanation, the optimum combination of pasteurization temperature and TG milk treatment ratio that gave the highest yield was (90 C°/ 15) combined with TG ratio of (3 gr TG/ kg milk).

4.1.3. Physicochemical Changes During Storage Time.

4.1.3.1. pH and Titratable Acidity.

During storage time of concentrated yogurt samples (Labneh), significant changes were observed on pH and titratable acidity results from the control Labneh sample (Fig.4.5). pH values significantly decreased during storage time (4.27± 0.02, 4.11±0.01, and 3.97± 0.03, respectively). While, titratable acidity values increased significantly during storage time (2.34±0.09, 2.52±0.09, and 3.12±0.03, respectively).

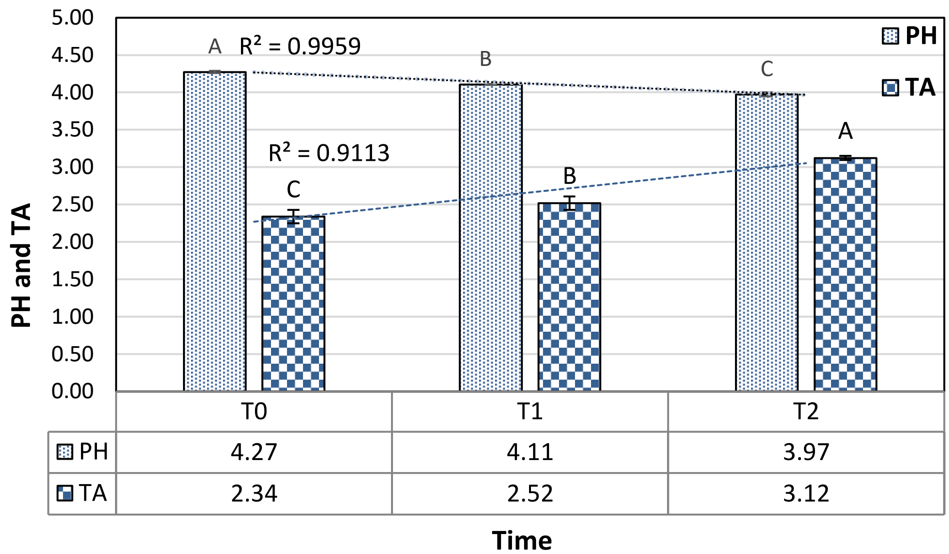


Fig.4.5: Changes on pH and Titratable acidity values of the control Labneh sample during storage time. ± Letters (A - D) indicate the statistical differences (at 0.05 level, Fisher test) between different storage periods for control Labneh sample.

The same pattern showed on TG samples results. Significant change on pH and titratable acidity results during time (Fig.4.6). pH values significantly decreased during storage time (4.32±0.03, 4.20±0.04, and 4.05, respectively). On contrary, the titratable acidity values increased significantly during time (1.33±0.09, 1.61±0.12, and 1.82±0.04, respectively).

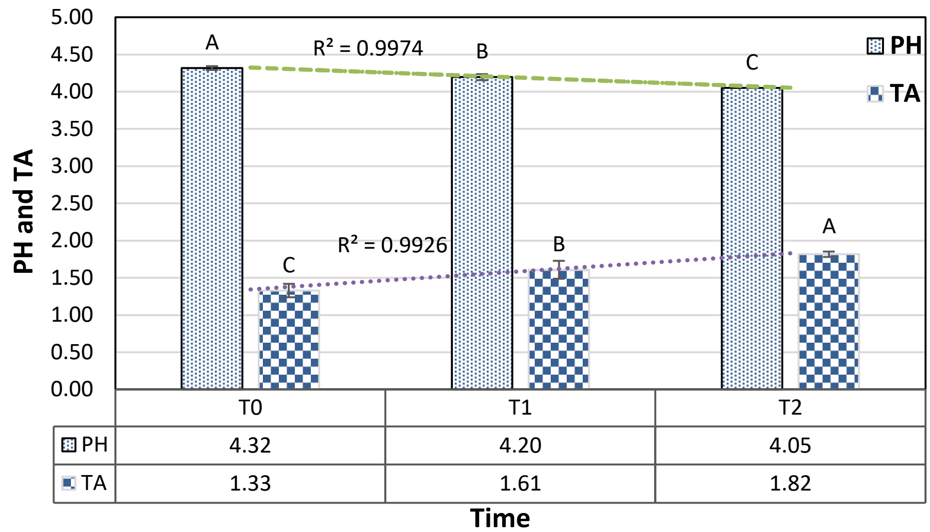


Fig.4.6: Changes on pH and Titratable acidity values of the TG Labneh sample during storage time. ± Letters (A - D) indicate the statistical differences (at 0.05 level, Fisher test) between different storage periods for TG Labneh sample.

A decrease in pH values and a corresponding increase in titratable acidity were exhibited by the control and TG samples during time. This can be explained by the activity of lactic acid bacteria (LAB), which break lactose and other sugars into lactic acid leading to pH decrease and titratable acidity increase (Wang et al., 2021). This explanation in agreement with Al-Kadamany et al., (2003) who all found that pH decreased and titratable acidity increased upon storage of Labneh as a result of LAB activity.

The low pH with cold storage, might allow yeast and mold growth (Setyawardani et al., 2022).

Comparing the pH and titratable acidity values of the control and TG sample, the TG treated samples pH values slightly higher than that of the control sample, and the titratable acidity values of TG sample lower than that of the control sample; this is due to the TG crosslinking that reduce the availability of low molecular weight (LMW) peptides, and thus reduce the growth of LAB (Lorenzen et al., 2002; Özer et al., 2007; Han et al., 2003).

These findings agreed with Özer et al. (2007) who found that enzyme lead to imbalance of the symbiotic growth of the yogurt culture. Lorenzen et al. (2002); and Özer et al. (2007) all of them found that MTGase reduce the growth of yogurt cultures, through the crosslinking formation, which induce longer lactic fermentation and slower acidity development process.

4.1.3.2. Syneresis and WHC.

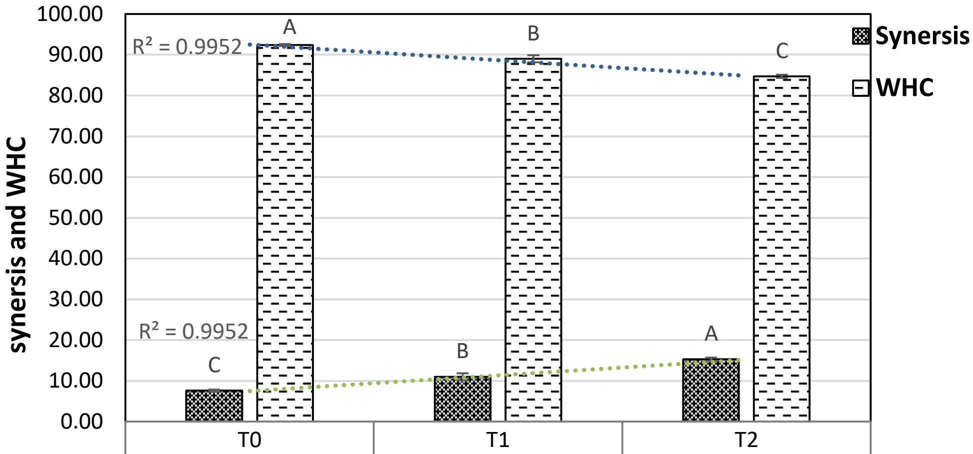
Curd syneresis is expressed as the watery part of the gel curd (free whey), that is released spontaneously due to the gel contraction (Dejmek, P., & Walstra, P., 2004). Whilst, WHC of the yogurt curd indicates the ability of the yogurt curd to hold or retain water in its gel (Sumarmono et al., 2019).

Results showed that the syneresis of the control Labneh sample significantly increased during storage time (Fig.4.7). The results were (7.63±0.22, 11±0.85, and 15.31±0.39, respectively). However, WHC results of the control sample decreased significantly during time (Fig.4.7), the results were, (92.38±0.22, 89±0.85, and 84.69±0.39, respectively).

Syneresis results of TG Labneh sample increased significantly up to (15 days) of storage (Fig.4.8). The result at (T0 was 5.04±1, and at T1 was 8.38±0.53). There was no significant increase in syneresis after (15 day) of storage time, as the result at (T2 was 9.59±0.02).

On contrary, WHC results of TG Labneh sample decreased significantly up to (15 days) of storage (Fig.4.8). The WHC value at (T0 was 94.97±1, and at T1 was 91.62±0.53). There is no significant decrease in WHC after

(15 day) of storage time, as the result at (T2 was 90.41±0.02).



	T0	T1	T2
Synersis	7.63	11.00	15.31
WHC	92.38	89.00	84.69

Fig.4.7: Changes on Syneresis and WHC values of control Labneh sample during storage time. ± Letters (A - D) indicate the statistical differences (at 0.05 level, Fisher test) between different storage periods for control Labneh sample.

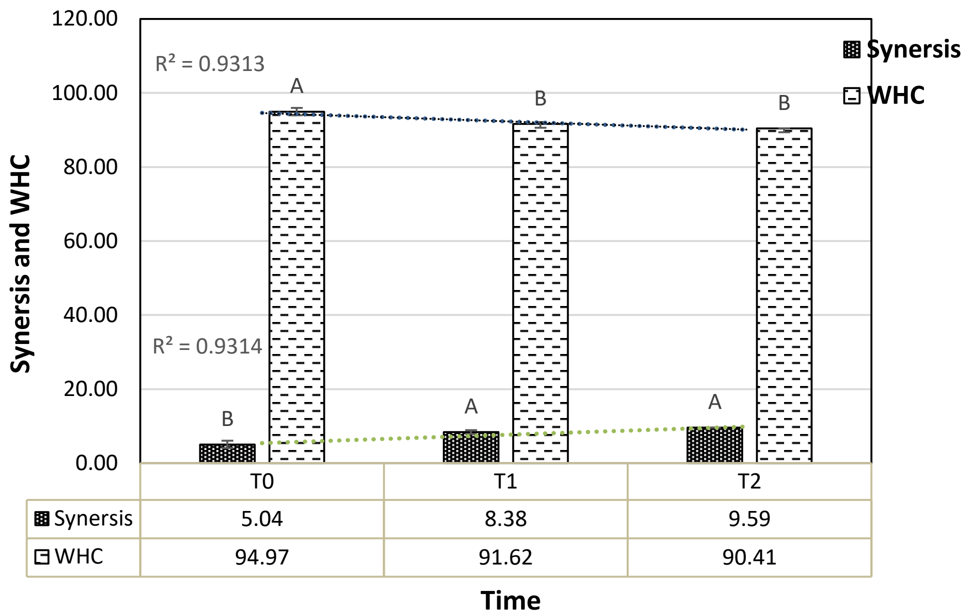


Fig.4.8: Changes on Syneresis and WHC values of TG Labneh sample during storage time. ± Letters (A - D) indicate the statistical differences (at 0.05 level, Fisher test) between different storage periods for TG treated Labneh sample.

The obtained results (Fig.4.7, Fig. 4.8) indicated that syneresis value increased during storage time, while WHC value decreased. This occurs due to the gel network contraction with pH decrease and TA increase during storage time (Jambi, 2018), and the subsequent rearrangement of the particles that making up the gel network (Van Vliet and Walstra, 1994; Lucey, 2002).

Comparing the results of the control Labneh sample with that of TG Labneh sample, it was noted that TG Labneh sample showed less increase in syneresis values, and less decrease in WHC values during storage time. However, these finding s might be explained by ty the action of TG crosslinking, which reduce LMW peptides availability, thus reduce LAB activity leading to lower acid development (Lorenzen et al., 2002; Özer et al., 2007; Han et al., 2003). Lower acidity enhances lower gel network contraction, and thus less syneresis occur (Guzmán-González et al., 2000).

The influence of TG on syneresis and WHC agreed with the findings published by Şanlı et al., (2011) who reported that TG enzyme addition after pasteurization enhanced gel stability with the reduction of syneresis. Abdulqadr et al., (2014); Cancino et al., (2006) who found that the increase in MTGase concentration resulted in syneresis decrease and WHC improvement. Lorenzen et al., (2002) who reported that MTGase crosslinking lower susceptibility to syneresis due to pore size differences of the gel. As higher level of syneresis (wheying-off) is explained by the larger pore size, since larger pores present a lower resistance to the outflow of whey from the gel (Aichinger et al., 2003). Guzmán-González et al., (1999); Guzmán-González et al., (2000) they showed that syneresis percentage was directly related to TA value and inversely to pH value change.

4.1.4. Microbiological Changes during Storage Time

4.1.4.1. Lactic Acid Bacteria (LAB) Growth during Storage Time.

During (30 day) of storage, the lactic acid bacteria of the control concentrated yogurt sample significantly decreased (Fig.4.9).

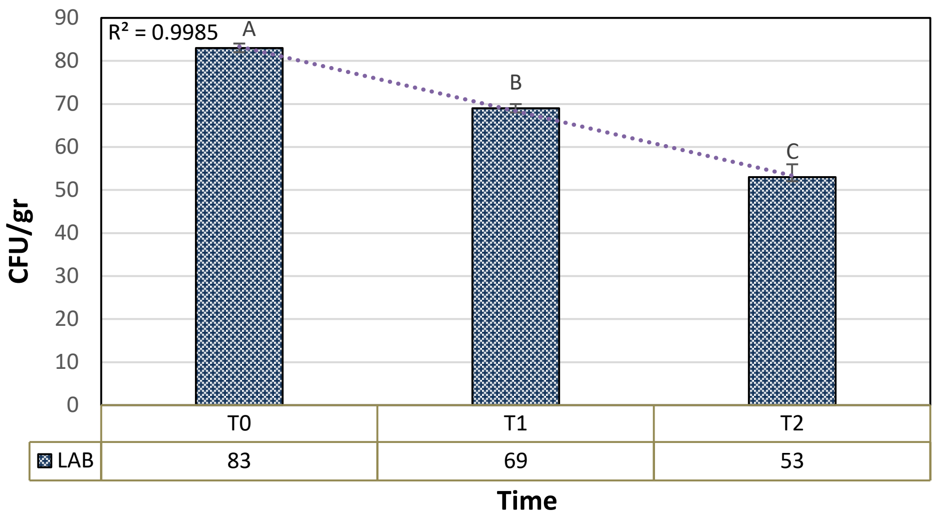


Fig.4.9: Changes on LAB growth of control Labneh sample during storage time. ± Letters (A - D) indicate the statistical differences (at 0.05 level, Fisher test) between different storage periods for control Labneh sample.

The average of LAB at (T0 was 83±1.00, then decreased gradually to 69±1.00, and 53±3.00, at T1, and T2), respectively.

The LAB growth of TG concentrated yogurt showed a sharp significant decrease during storage time (Fig.4.10). The average of LAB at (T0, T1, and T2 were 74±2.00, 39±1.00, and 16.67±1.53), respectively.

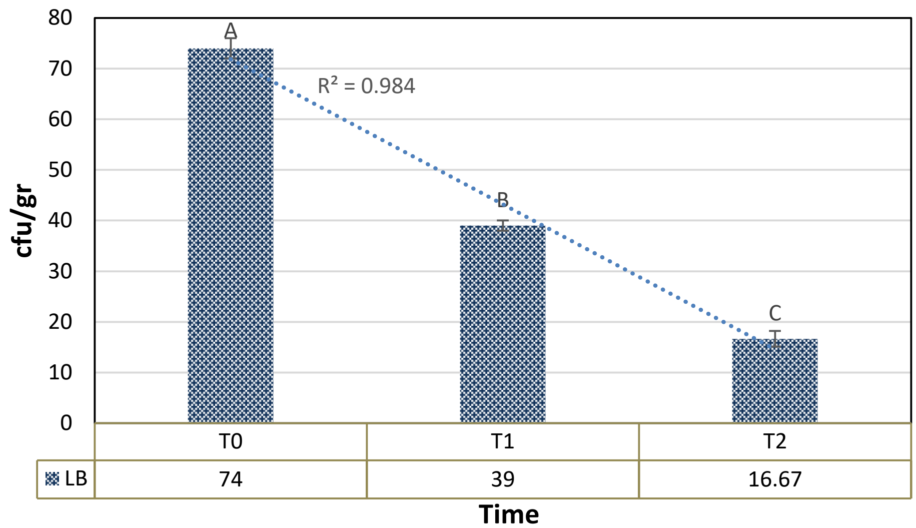


Fig.4.10: Changes on LAB growth of TG Labneh sample during storage time. ± Letters (A - D) indicate the statistical differences (at 0.05 level, Fisher test) between different storage periods for TG treated Labneh sample.

Generally, LAB growth decreased during storage time. The growth of LAB was affected by culture medium pH. As a result, when the PH value decrease below (4.5) the LAB growth decrease (Vera-Peńa and Rodriguez Rodriguez, 2020).

By comparing the decrease in LAB growth for control and TG concentrated yogurt during storage time. TG sample showed higher decrease in LAB growth upon time, this can be explained by the effect of TG crosslinking. A symbiotic growth between the yogurt starter cultures, in the first step, S. thermophilus fermentation of lactose produces lactic acid, folic acid, formic acid, CO_2, etc. These chemicals promote the growth of L. bacillus (Aswal et al., 2012). In the second step, the proteolytic lactobacilli by liberating amino acids and small peptides provide the most important growth promoting sources for S. thermophilus (Aswal et al., 2012). TG enzyme form protein crosslinking, that crosslink LMW peptides and/or amino acids that are required by S. thermophilus activity. As S. thermophilus substrate became un-available the starter culture bacteria growth declined (Lorenzen et al., 2002; Özer et al., 2007; Han et al., 2003).

Similar conclusions were drawn by Faergmand et al., (1999); Özer et al., (2007) who reported that LAB growth rate was reduced in the presence of TG, dependent on the enzyme concentration in the yogurt. Soleymanpuori et al., (2014) proposed a reduced growth rate for LAB at higher MTGase levels, as LAB have a lower accessibility to the specific nitrogen sites of the cross linked proteins, leading to a reduction of their survival rate.

On the other hand, Dinkci (2012) showed that no significant differences in the number of starter culture bacteria during storage period, for strained yogurt prepared with MTGase treated milk.

4.1.4.2 Yeast and Mold Growth during Storage Time.

In the control sample of concentrated yogurt, yeast and mold count exhibited a little slow increase during the first (15 days) of storage. Whereas, at the second (15 days) of storage, the count results exhibited a sharp significant increase (Fig.4.11).

Its noteworthy that yeast and mold were not detected in fresh control sample (T0); due to eradication of vegetative forms during heat treatment of raw milk and good hygienic conditions of processing.

At T1 of storage time counts increased a little with no significant difference. At the end of storage time (T2), the highest count average exhibited which was (29±2.65).

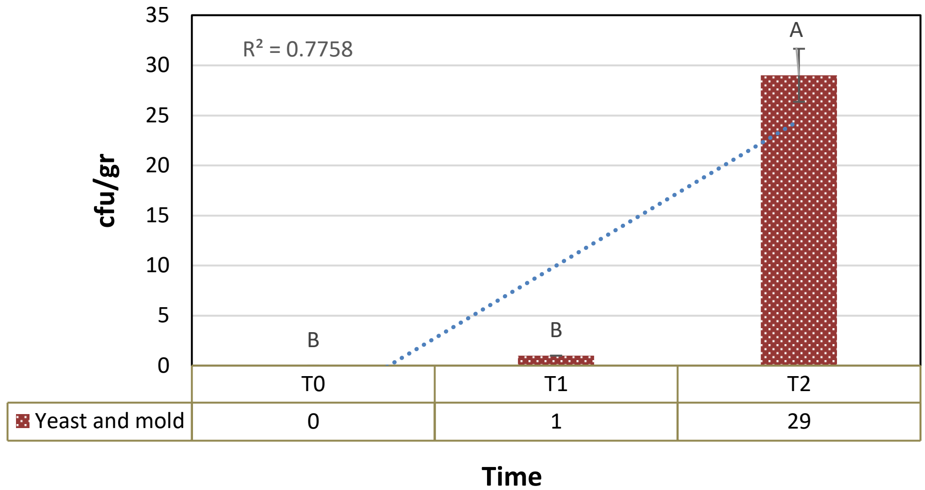


Fig.4.11: Changes on yeast and mold growth of control Labneh sample during storage time. ± Letters (A - D) indicate the statistical differences (at 0.05 level, Fisher test) between different storage periods for control Labneh sample.

For TG concentrated yogurt (Fig.4.12), yeast and mold count exhibited a little increase with no significant difference at the first (15 days) of storage. The count averages were (0.67±0.58, and 3.33±0.58), at (T0) and (T1) respectively.

After (15 days) of storage, the counts increased very sharply with significant difference. The count average at the end of storage time (T2) exhibited the highest value (183.67±44.50).

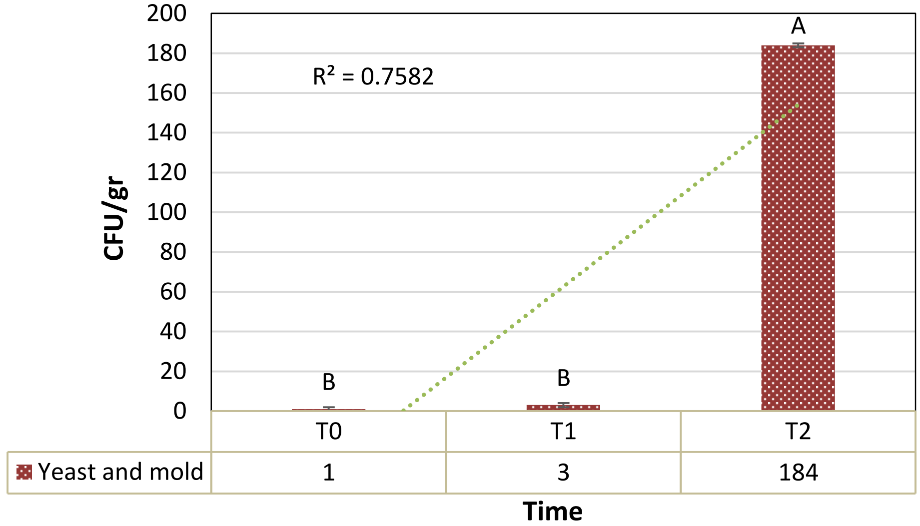


Fig.4.12: Changes on yeast and mold growth of TG Labneh sample during storage time. ± Letters (A - D) indicate the statistical differences (at 0.05 level, Fisher test) between different storage periods for TG treated Labneh sample.

According to the previously mentioned results, it can be concluded that:

1. Yeast and mold growth at the first (15) days of the cold storage were stable. These findings are in accordance with Uysal (1993); Kesenkaş (2010); and Dinkci (2012).

2. Yeast and mold growth increased significantly after (15) days of storage. Strained yogurt is suitable environment for yeast and mold growth; because of its low pH, and limited access to air at packaged, refrigerated storage conditions. At ambient temperatures (20-25 C°), deterioration of Labneh can be rapid but, even at (7C°) the characteristic signs of yeast spoilage often become visible in (7- 14) day (Yamani and Abu-Jaber, 1994).

Comparing the sharp increase of yeast and mold counts for control and TG concentrated yogurt after (15) days of cold storage. The TG sample exhibited higher average (183.67±44.50) than the control sample

(29±2.65).

The patterns of change in titratable acidity, pH, and WHC of concentrated yogurt upon storage time correlated with yeast and mold growth stimulation. Moisture content is known to be one of the most critical components in promoting mold growth (Block, 1953). TG concentrated yogurt showed higher WHC upon storage than the control. Thus, beside the low pH, higher WHC will facilitate greater growth.

The observed growth of yeast and mold at T2 of storage time considered as failure criteria for cloth bag Labneh. As the yeast and mold break down peptides (proteolytic activity), sensorial defects (off flavors), and textural defects detected. Therefore, Antifungal needed to control yeast and mold growth (Collins and Moustafa, 1969; Mihyar et al., 1999).

4.2. Local White- Brined Cheese (WBC).

4.2.1. Influence of Different Pasteurization Temperatures, combined with Different Milk TG Treatments on the Yield Percentage of the Local White- Brined Cheese.

The obtained results of each pasteurization temperature will be discussed separately according to the added TG enzyme concentrations, and presented in figures for better illustration.

4.2.1.1. Influence of (65 C°/ 30 min) Pasteurization Temperature combined with Different Milk TG Treatments on the Yield Percentage of the Local White- Brined Cheese.

At (65C°/ 30 min) pasteurization temperature, yield percentage of WBC increased significantly when the used milk treated with TG enzyme

(Fig.4.13).

In respect to the control white brined cheese (Z sample) with yield percentage (13.65± 0.01), the yield percentages of TG treated WBC samples as follows (16.95±0.01) (A sample), (15.44±0.01) (B sample), and (16.21±0.01) (C sample).

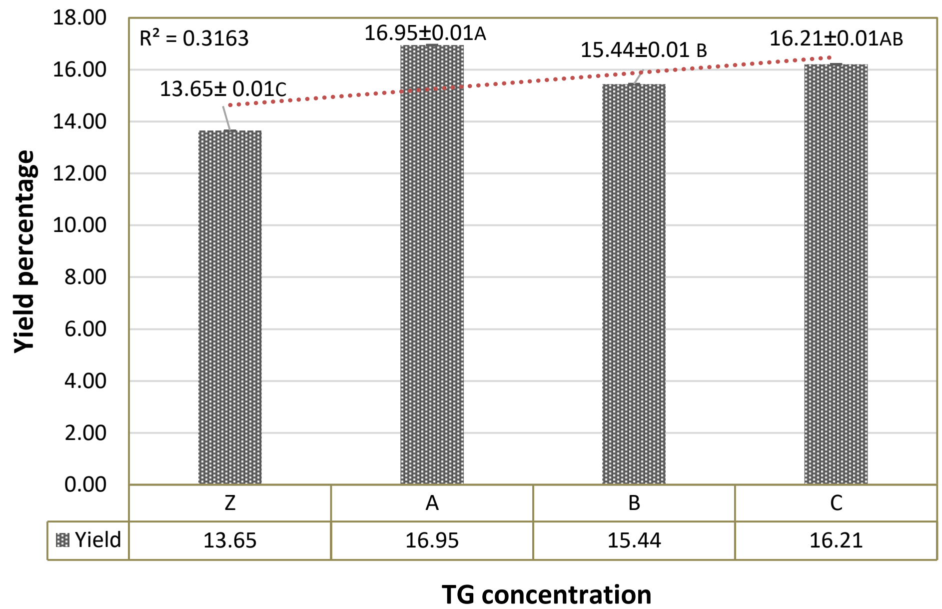


Fig.4.13: Influence of (65 C°/ 30 min) pasteurization temperature combined with different milk TG treatments on the local WBC yield percentage. ± Letters (A - D) indicate the statistical differences (at 0.05 level, Fisher test) between different TG treated WBC samples.

The highest yield percentage obtained when the milk treated with (1 gm TG/ kg milk) ratio, followed by (5 gm TG/ kg milk) ratio, and the lowest was (3 gm TG/ kg milk) ratio.

According to the previously mentioned results, it can be concluded that the optimum TG ratio that give the highest WBC yield percentage, at (65 C°/ 30 min) pasteurization temperature was (1 gm TG/ kg milk).

4.2.1.2. Influence of (72 C°/ 15) Pasteurization Temperature combined with Different Milk TG Treatments on the Yield Percentage of the Local White- Brined Cheese.

At (72 C°/ 15 sec) pasteurization temperature, the yield percentage of WBC significantly increased when the used milk treated with TG (Fig.4.14).

The control WBC yield percentage was (18.65±0.01). However, the yield percentages of A sample (1gm TG/ kg milk), B sample (3gm TG/ kg milk), and C sample (5gm TG/ kg milk) were as the following: (21.36±0.02, 24.58±0.01, and 23.38±0.01), respectively.

Significant difference appeared between the yields of TG treated samples. B sample showed the highest yield percentage, followed by C sample, and the lowest yield percentage showed by A sample.

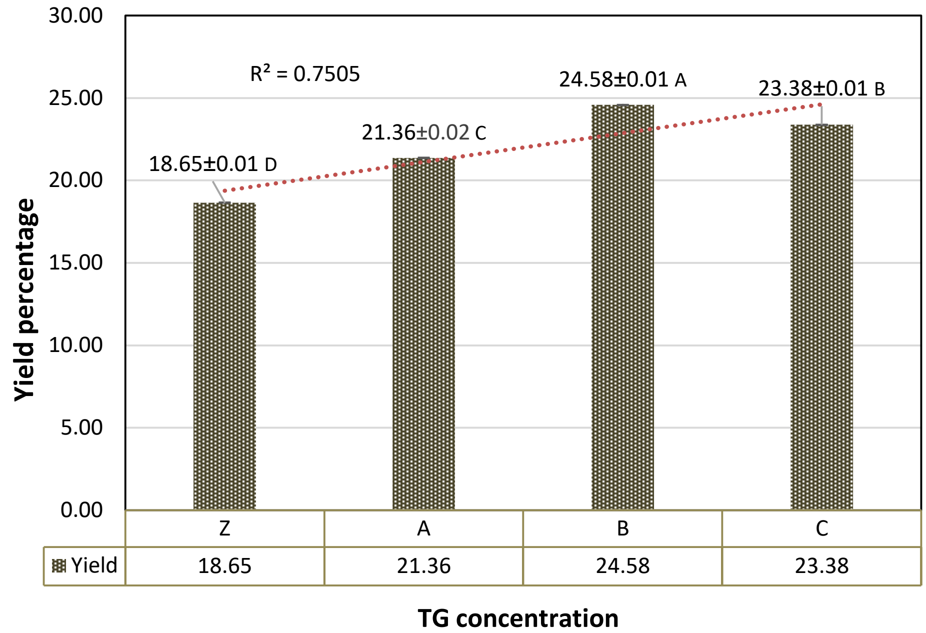


Fig.4.14: Influence of (72 C°/ 15 sec) pasteurization temperature combined with different milk TG treatments on the local WBC yield percentage. ± Letters (A - D) indicate the statistical differences (at 0.05 level, Fisher test) between different TG treated WBC samples.

Based on the previously mentioned results, it can be summarized that, at (72 C°/ 15) pasteurization temperature, the optimum TG ratio that gave the highest WBC yield percentage was (3gm TG/ kg milk).

4.2.1.3. Influence of (82 C°/ 15) Pasteurization Temperature combined with Different Milk TG Treatments on the Yield Percentage of the Local White- Brined Cheese.

At (82 C°/ 15) pasteurization temperature, findings obtained showed significant increase in the yield percentage of WBC treated with TG compared to the percentage obtained from the control WBC (Fig.4.15).

The yield percentage of the control WBC was (17.61±0.01). However, the yield percentages of WBC treated with (1gm TG/kg milk) (A sample), (3 gm TG/kg milk) (B sample), and (5 gm TG/kg milk) (C sample) ratios were as the following: (23.03±0.02, 25.95±0.01, and 25.31±0.01), respectively.

Comparing the yield percentages of TG-WBC treated samples, significant increase was obtained as the TG ratio increased from (1gm TG/kg milk) to (3 gm TG/kg milk).

Depending on the previously mentioned results, it can be determined that, the optimum TG ratio that gave the highest WBC yield percentage, when (82 C°/ 15) pasteurization temperature applied, was (3 gm TG/kg milk).

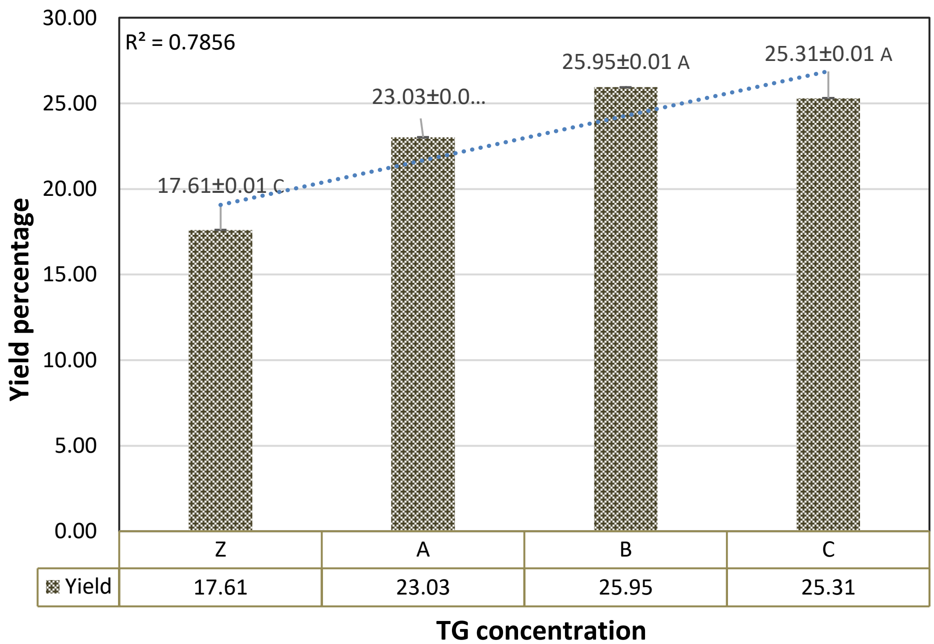


Fig.4.15: Influence of (82 C°/ 15 sec) pasteurization temperature combined with different milk TG treatments on the local WBC yield percentage. ± Letters (A - D) indicate the statistical differences (at 0.05 level, Fisher test) between different TG treated WBC samples.

4.2.2. Comparison between the Highest Yield Samples at the Different Pasteurization Temperatures.

The WBC samples that gave the highest yield percentages at the different pasteurization temperatures with optimum TG ratio were showed in table (4.2).

Table 4.2: The highest yield local white- brined cheese samples at the different pasteurization temperatures with optimum TG ratio. ± Letters (A - D) indicate the statistical differences (at 0.05 level, Fisher test) between different TG treated samples.

Pasteurization temperature	Sample with highest yield %	Sample TG ratio	Yield %
65 C°/ 30 min	A	1 gm TG/ kg milk	16.95±0.01A
72 C°/ 15 sec	B	3 gm TG/ kg milk	24.58±0.01 A
82 C°/ 15 sec	B	3 gm TG/ kg milk	25.95±0.01 A

In comparison between the yield percentages of WBC at different pasteurization temperatures, the results indicated that the yield percentages were highly affected by the pasteurization temperature. In other words, as the pasteurization temperature increase, WBC yield percentage increase.

The positive correlation between the pasteurization temperatures and the optimum TG treatments that gave the highest yield percentages can be explained by:

1. Influence of heat treatment (HT) on milk proteins.

Upon heating milk above (65 C°), whey proteins are denatured by unfolding of their polypeptides, thus exposing the side chain groups originally buried within the native structure. The unfolded proteins then interact with the casein micelles, or simply aggregate with themselves, involving thiol-disulphide interchange reactions, hydrophobic interactions, and ionic linkage. (Sigh and Waungana, 2001).

Higher pasteurization temperatures or longer holding times would cause more heat induced interactions of casein and whey proteins (Lau et al., 1990). The presence of whey proteins allowed higher moisture retention, due to their hydrophilic property (Meinardi et al., 2003).

The positive correlation between the heat treatment and cheese yield also approved by several previous studies on various cheese types. Lau et al., (1990) who investigate the influence of pasteurization temperature (63C°/ 30 min) on Cheddar cheese yield, and on fat and nitrogen (i.e., protein) recovery in cheese. Pasteurization had no effect on fat recovery in cheese, but nitrogen recovery was higher for cheese made from pasteurized milk. The higher nitrogen recovery was appeared due to whey protein (approximately 5% of milk whey proteins) association with casein micelles after milk pasteurization. It was concluded that heat denaturation of whey protein caused by milk heat treatment results in Cheddar cheese yield increase.

Abdou and Dawood, (1977); Abou-Dawood and Gomai, (1977) found that the yield percentage of Kariesh cheese was higher in heated milk cheese than unheated milk cheese.

Ghosh et al., (2000) who compared the Camembert- type cheese yield at different heat treatments of cheese milk. Cheese was manufactured from pasteurized milk (72 C°, 15 sec), heated milk (80 C°, 30min), and a (30:70) mixture of high heated milk (90 C°, 6 min) and raw milk. Yield, moisture retention, solids and protein recovery significantly increased in the cheeses produced from heated and mixed milk both with a total denaturation degree of (30%) of whey proteins.

Şimşek and Sagdic, (2012) who reported that the yield of Çökelek cheese heated at (95 C°) was higher than of cheese heated at (85 C°), which was due to higher whey protein denaturation.

2. Influence of heat treatment on TG- induced reactions.

TG crosslinking of milk proteins is determined by the molecular structure and accessibility of glutamine and lysine residues (Sharma et al., 2001).

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Casein proteins can be easily cross linked by TG enzyme, due to the high flexibility, absence of disulfide bonds, along with little or no secondary protein structure (Sharma et al., 2001). In comparison to the whey proteins with globular dense structure stabilized by disulfide bridges (Sharma et al., 2002). Therefore, globular whey protein requires steps that promote their partial denaturation to make them accessible to the enzymatic activity (DeJong and Koppelman, 2002; Bonisch et al., 2007).

Milk proteins more susceptible to TG crosslinking when milk undergo heat treatment. As the HT affects the protein structure and causes unfolding that led to protein interactions. These reactions appear to enhance susceptibility of milk proteins to TG reactions (Sharma et al., 2001).

3. Influence of TG crosslinking on cheese yield.

Several studies found that the TG crosslinking induce higher cheese yield. Pierro et al., (2010) who reported that TG is able to improve cheese yield by increasing curd water retention. As the TG led to gel matrix formation with lower pore size, resulting in decreasing syneresis and retaining more serum in the gel.

Özer et al., (2013) who revealed that protein crosslinking by MTGase was an effective tool to obtain White- brined cheese with modified textural characteristics and increased yield. The increase in cheese yield was not due to more serum retention alone, but also to slight increase in relative protein level of MTGase treated cheese.

Garcia- Gómez et al., (2019); Bönisch et al., (2008) found that White cheese yield increased by the addition of TG. This increase due to the increase in the protein and moisture content of cheese. TG increased the protein content by providing covalent bonding between protein molecules, and increased moisture content by increasing of serum binding in the gel structure, which increased the production yield.

Based on the previously mentioned findings, it can be deduced that the WBC yield percentage highly influenced by pasteurization temperature and TG treatments of cheese milk. So, as the pasteurization temperature increased, the level of whey protein denaturation increased, and thus whey protein susceptibility for TG crosslinking increased, followed by increase in moisture retention, which reflected on yield increase. That provide an explanation for the relationship between pasteurization temperature, TG ratio, and the highest WBC yield percentage.

At (65 C°/ 30 min) pasteurization temperature, the optimum TG ratio that gave the highest yield percentage was (1 gr TG/kg milk). However, at higher pasteurization temperatures (72 C°/ 15 sec, and 82 C°/ 15 sec) TG ratio that gave the highest yield percentages was (3 gr TG/ kg milk).

At (65 C°/ 30 min) pasteurization temperature, the level of whey protein denaturation is lower than that of the other heat treatments. So, it's thought that (1 gr TG/kg milk) ratio the optimum to crosslink the available susceptible proteins, and increasing TG ratio will not give more yield percentage increase.

As a conclusion based on the previously mentioned explanation, the optimum combination of pasteurization temperature and TG milk treatment ratio that gave the highest yield was (72 C°/ 15 sec) combined with TG ratio of (3 gr TG/ kg milk).

Milk pasteurization at (72 C°/ 15 sec) was preferred over (82 C°/ 15 sec) pasteurization temperature; due the formation of weaker and finer curd, which retains more water, as the milk heated at higher temperatures.

The finding of preferring (72 C°) pasteurization temperature of cheese milk, was in agreement with Patel et al., (1986) who found that heat treatment of Mozzarella cheese milk (72 C°, no hold), improved protein and total solids (TS) recovery, but decreased the fat recovery, gave soft bodied cheese, improved flavor score, keeping quality, and ensured public health safety.

Beeby et al., (1971) found that heat treatment greater than pasteurization (i.e., 72 C°/ 16 sec) hasn't been favored for production Cheddar cheese because of slower coagulation, weaker curd, and impaired syneresis it produced. However, such milk has been used to increase yield of Cottage cheese, since it a fresh cheese with acidic coagulation (Kannan and Jenness, 1956).

4.2.3. Physicochemical Changes during Storage Time.

4.2.3.1 pH and Titratable Acidity during Storage Time.

Upon storage time, pH and titratable acidity of the control WBC showed significant changes (Fig. 4.16, and Fig. 4.17).

The pH values of the control WBC showed a steady significant decline from (T0) until (T2), from an initial pH of (7.48±0.02 to 7.22±0.01).

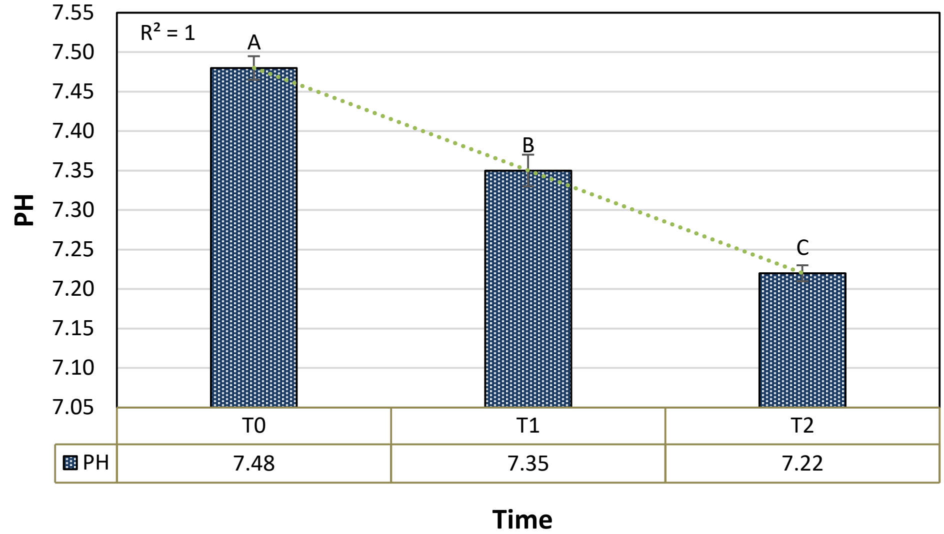


Fig.4.16: Changes on pH values of the control white brined cheese during storage time. ± Letters (A - D) indicate the statistical differences (at 0.05 level, Fisher test) between different storage periods of control WBC sample.

The titratable acidity values of the control WBC were found to increase significantly as the storage period progressed. The lowest TA value showed at (T0) (fresh WBC sample), which was (0.23±0.02). The TA values continued a significant increase upon storage time. The values of TA at (T1) and (T2) were (0.28±0.01, and 0.37±0.01), respectively.

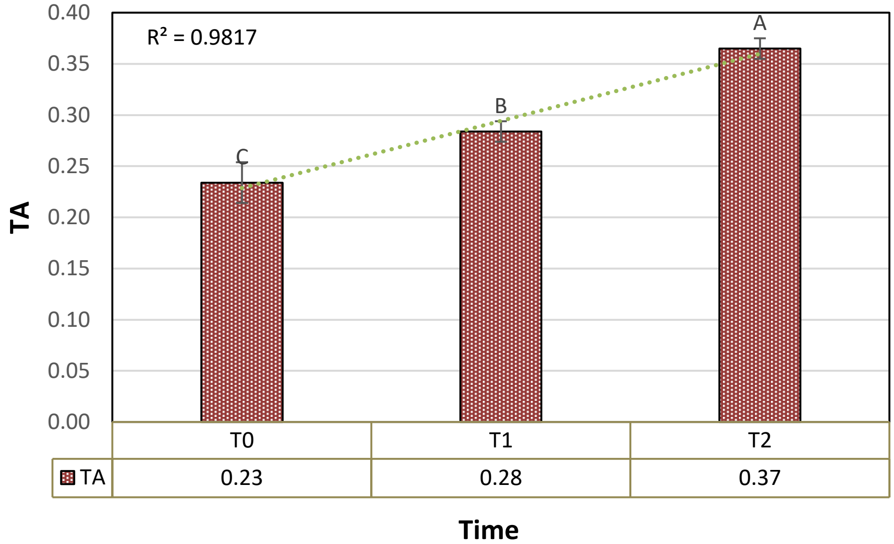


Fig.4.17: Changes on TA values of the control white brined cheese during storage time. ± Letters (A - D) indicate the statistical differences (at 0.05 level, Fisher test) between different storage periods of control WBC sample.

The pH and TA values of TG white brined cheese are also affected by the storage duration as indicated in Fig. (4.18), and (4.19).

A steady significant decline was exhibited for the pH of TG white brined cheese. The initial pH at (T0) showed the highest value which was (7.66±0.01), then a significant decline followed at (T1) and (T2), with pH values of (7.38±0.01, and 7.27±0.01), respectively.

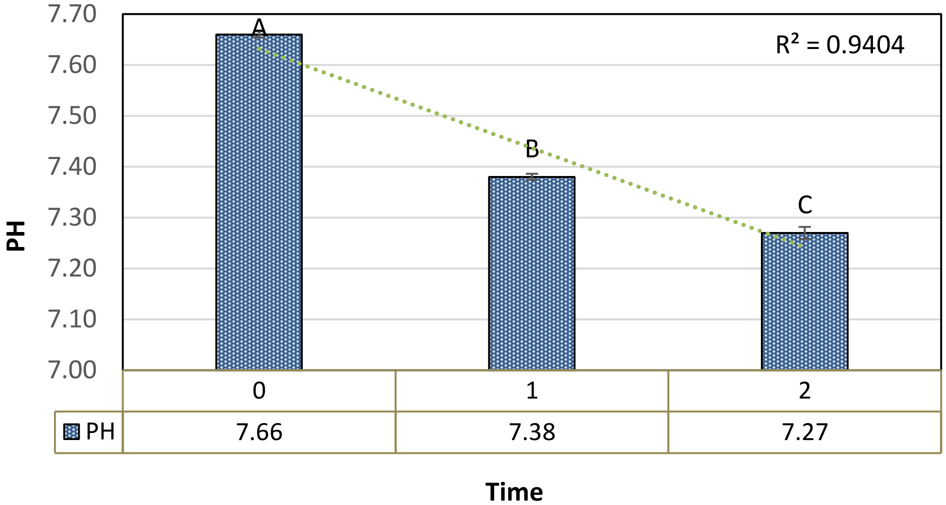


Fig.4.18: Changes on pH values of the TG white brined cheese during storage time. ± Letters (A - D) indicate the statistical differences (at 0.05 level, Fisher test) between different storage periods of TG treated WBC sample.

On the contrary, the TA values of TG white brined cheese showed a significant increase as the storage time progressed.

The initial TA at (T0) showed the lowest value, which was (0.14±0.01), then a significant increase followed at (T1) and (T2), with a values of (0.23±0.03, and 0.30±0.02), respectively.

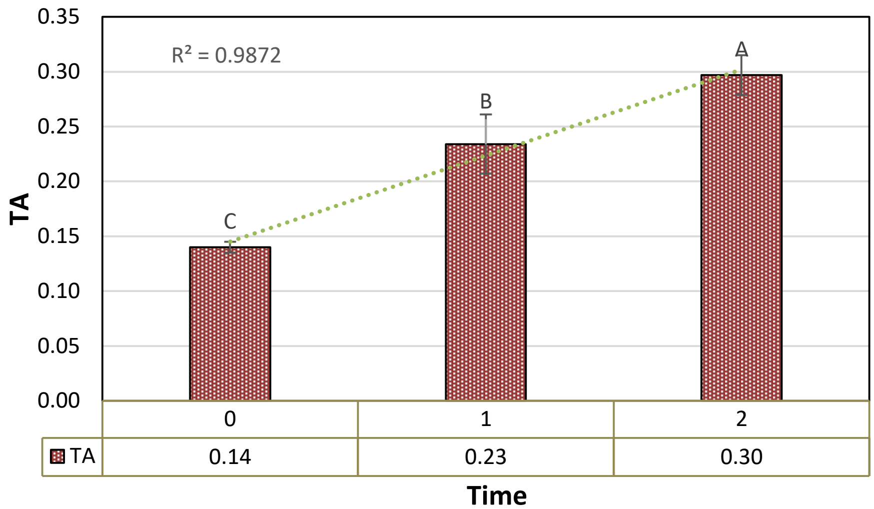


Fig.4.19: Changes on TA values of the TG white brined cheese during storage time. ± Letters (A - D) indicate the statistical differences (at 0.05 level, Fisher test) between different storage periods of TG treated WBC sample.

In general, the pH values decreased and TA values increased as the storage time progressed.

The pH findings agreed with Lazárková et al., (2021); Memiši et al., (2014) who revealed that the pH values of WBC decreased with increasing of the storage temperature and with the prolonging of the storage time.

The obtained TA were in accordance with Eljagmani and Altuner, (2020); Ceylan et al., (2007); Kesenkaş et al., (2012); Revilla et al., (2007) who found similar TA changes in different varieties of cheese.

The increase in acidity upon storage period was mainly due to the action of lactic acid bacteria that convert lactose to lactic acid (Bilal, 2000; Warsama et al., 2006; Walstra et al., 1999; El Owni and Hamid, 2008; Hayaloglu et al., 2005).

The microbiota of raw milk includes numerus strains of non- starter lactic acid bacteria (NSLAB), its play important role during cheese ripening, and flavor development (Settanni and Moschetti, 2010).

Comparing pH and TA results obtained from the control and TG white brined cheese. The pH values of TG- WBC during time were slightly higher than the control WBC values. However, the TA values of TG-WBC during time less than that of the control sample.

This can be explained by the action of TG crosslinking. The TG crosslinking reduce the availability of LMW peptides, and thus reduce the growth of LAB (Lorenzen et al., 2002; Özer et al., 2007; Han et al., 2003).

The obtained lower acidity results of TG white brined cheese agreed with Dmytrow et al., (2010) who reported that an increase in enzyme concentration could lead to a delay in the bacterial reproduction, and could lead to slower increase in acidity.

Pham et al., (2021) suggested that enzyme addition to milk could cause imbalances in the growth of lactic acid bacteria by crosslinking reaction.

4.2.3.2 Moisture and Dry Matter Contents during Storage Time.

The obtained moisture and dry matter results of the control WBC showed a non- significant change upon storage time (Fig.4.20).

It was marked that moisture results of the control WBC underwent a non-significant decline as the storage time progressed. The highest value showed at (T0) which was (53.99±0.55). Moisture value declined to (51.57±4.27) at (T1), and at the end of storage time (T2) the moisture value was (50.08±5.43).

On the other side, DM results of the control WBC underwent a non-significant increase as the storage time progressed. The lowest value showed at (T0) which was (46.02±0.55). DM value increased to (48.43±4.27) at (T1), and at the end of storage time (T2) the highest DM value found which was (49.92±5.43).

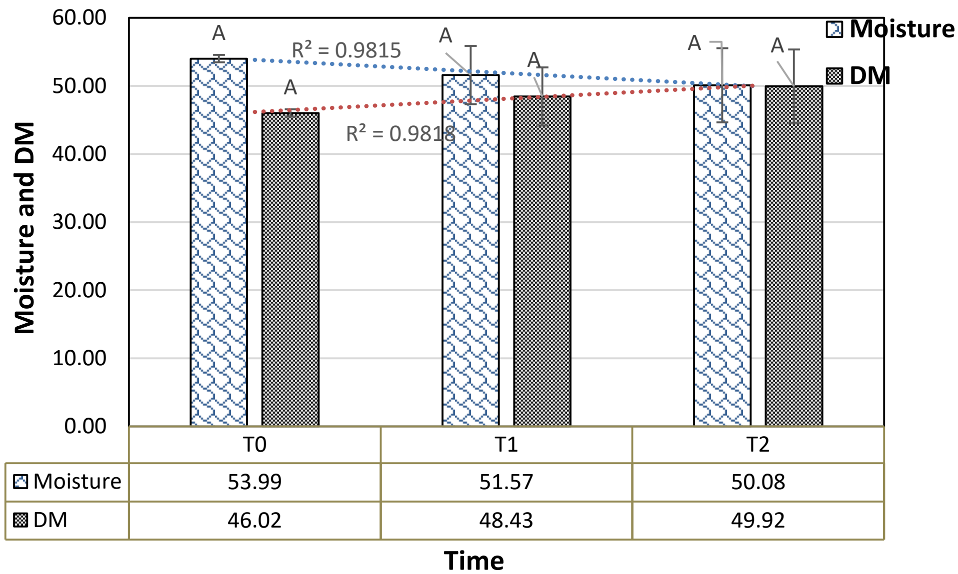
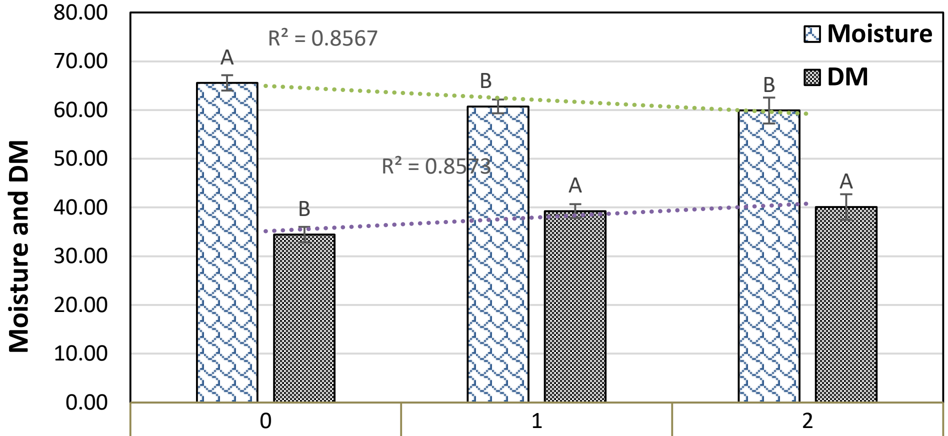


Fig.4.20: Changes on Moisture and DM contents of the control white brined cheese during storage time. ± Letters (A - D) indicate the statistical differences (at 0.05 level, Fisher test) between different storage periods of control WBC sample.

The obtained moisture and DM results of TG-WBC showed a significant change at the first (15) day of storage time, and non-significant change at the second (15) day of storage (Fig.4.21).

The TG- WBC moisture results exhibited a significant decline during the first (15) day of storage. The highest value obtained at (T0), which was (65.59±1.57), then significant decline showed at (T1), as the moisture value was (60.73±1.41). As the storage time developed reaching (T2), moisture value exhibited a non-significant decline, as the value was (59.90±2.64).

On contrary, DM results of TG- WBC showed a significant increase as the time progressed during the first (15) day of storage. At (T0), the lowest DM found, which was (34.44±1.56). DM value significantly increased reached a value of (39.27±1.41), at (T1) of storage period. The highest value obtained at (T2), as the DM value showed a non-significant increase, which was (40.10±2.64).



	0	1	2
Moisture	65.59	60.73	59.90
DM	34.44	39.27	40.10

Fig.4.21: Changes on Moisture and DM contents of TG white brined cheese during storage time. ± Letters (A - D) indicate the statistical differences (at 0.05 level, Fisher test) between different storage periods of TG treated WBC sample.

In general, from the results mentioned above its clear that as the storage time progressed, WBC moisture content decreased and DM content increased.

The moisture content decrease upon storage was similar to the findings reported by Bilal (2000); and Nuser (2001) who found that moisture move from the cheese to the pickling solution continuously, which increase cheese weight loss.

Aly and Galal (2002) revealed that the moisture content decreased in all cheeses throughout the storage period.

Zaki et al., (1975); Nofal et al., (1981) concluded that the cheese moisture content decrease during storage, is attributed to curd contraction and whey expulsion, as a result of acid development.

The increase of cheese DM during storage may be attributed to the moisture loss upon time. Aly and Galal (2002); El Owni and Hamid (2008); postulated similar justification. However, the obtained findings were in disagreement with Dariani et al., (1980); Hayaloglu et al., (2005) who reported total solid content decreasing; due to degradation of total protein, and fat content decrease during storage period.

Comparing the obtained moisture and DM results of TG and control WBC, it can be deduced that the moisture content of TG sample during storage time was higher than the control sample. On the other hand, DM content of TG sample was remarkably lower than the control sample upon storage time.

The higher moisture content, and lower dry matter content of TG- WBC can be explained due to the crosslinking induced by TG enzyme.

The cross linked molecules formed by TG have increased the water holding capacity and the stability of the gel network. Casein micelles mainly formed the gel network structure, due to its high flexibility, simple structure and ease to be cross linked by TG enzyme (Chen et al., 2019). Furthermore, the casein micelles covalently bonded by TG were not dissociated during acidification. This inhibit the rearrangement of these molecules, which lead to whey separation minimization (Pham et al., 2021).

Several studies reported that the addition of microbial transglutaminase (MTGase) increase the moisture content in various chesses, including White- brined cheese (Özer et al., 2013), low- fat Cheddar cheese (Hu et al., 2013), low- fat Iranian white cheese (Sayadi et al., 2012, 2013), Edam cheese (Aaltonen et al., 2014), and Ultra-filtration (UF)- White soft cheese (Ibrahim et al., 2017).

The lower dry matter content of TG- WBC compared to the control sample, was mainly related to increased water retention capacity of the rennet gel formed with TG crosslinking. Similar findings reported by Farnsworth et al., (2006); and Lorenzen et al., (2002).

4.2.4. Microbiological Changes during Storage Time.

4.2.4.1. Total Bacterial Count (TBC) during Storage Time.

Total bacterial count in the control WBC increased with increase in storage time, as marked in Fig (4.22).

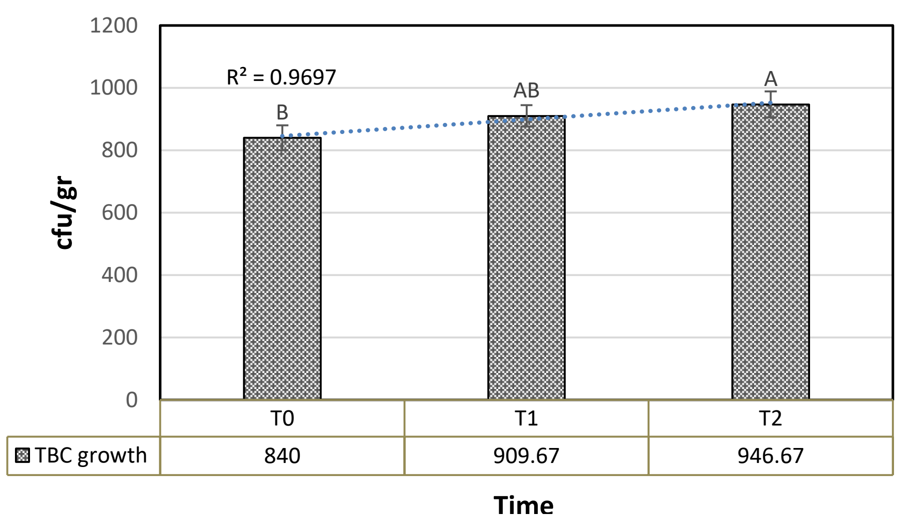


Fig.4.22: Changes on TBC of the control white- brined cheese sample during storage time. ± Letters (A - D) indicate the statistical differences (at 0.05 level, Fisher test) between different storage periods of control WBC sample.

At (T0), total bacterial count was (840±40.00), then a non- significant increase showed at (T1) and (T)2 of the storage time, the total bacterial count was (909.67±34.36, and 946.67±41.63), respectively. The highest count found at the end of storage time (at day 30).

Total bacterial count in TG- WBC increased significantly with increase in storage time, as marked in Fig (4.23).

A significant increase exhibited with storage time increase, from count of (508±47.52 at T0, to 664.67±60.47 at T1, and 886.67±90.19 at T2).

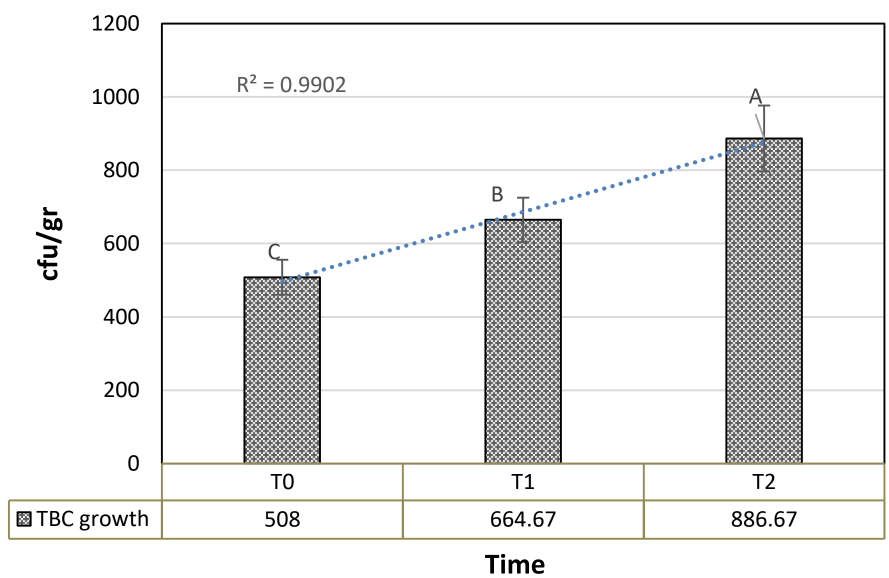


Fig.4.23: Changes on TBC of the TG white- brined cheese sample during storage time. ± Letters (A - D) indicate the statistical differences (at 0.05 level, Fisher test) between different storage periods of TG treated WBC sample.

The TBC results were in a line with those of Ahmed (1985); Aly and Galal (2002); El Owni and Hamid (2008), who reported that TBC count increase upon storage time. The increase possibly attributed to rapid growth of microorganisms during early stages of ripening.

Comparing the TBC count of the control WBC and TG- WBC during time, it can be deduced that the control WBC exhibited higher TBC increase than TG- WBC. This can be explained by the influence of TG crosslinking. As the crosslinks formation reduce the bioavailability of some necessary nutrients for microorganism's growth (Mahmood and Sebo, 2009; Han et al., 2003).

This finding was in agreement with Mahmood and Sebo (2009) who found that MTGase crosslinking led to cheese shelf life elongation, as the crosslinking reduce nutrients availability for the growth of deteriorative microorganisms. Han et al., (2003) who noticed retardation of lactic acid bacteria growth, and low acid formation, upon the cream cheese treatment with MTGase.

4.2.4.2. Total Coliform Growth during Storage Time.

The total coliform count of the control WBC significantly decreased as the storage time increased (Fig.4.24).

Total coliform count at (T0) was (74.67±8.51), then significantly decreased upon time to (48.33±4.73, and 30.67±2.89), at (T1) and (T2) respectively.

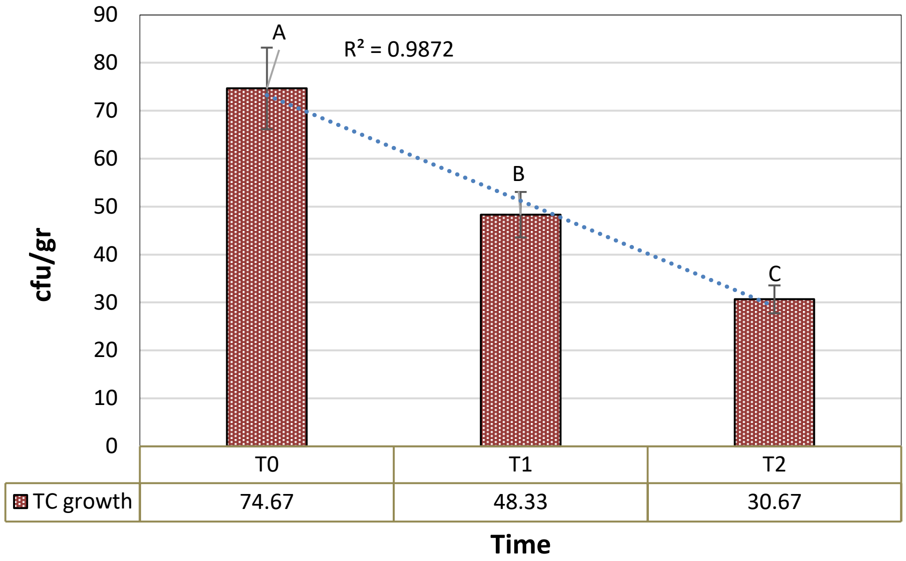


Fig.4.24: Changes on TC count of the control white- brined cheese sample during storage time. ± Letters (A - D) indicate the statistical differences (at 0.05 level, Fisher test) between different storage periods of control WBC sample.

The total coliform count of TG- WBC exhibited a significant decrease as storage time progressed (Fig.4.25).

At (T0) total coliform count was (25.33±3.51). Then, the total coliform count revealed a continuous decrease with time progress. At (T1) and (T2), the total coliform results were (17±3.00, and 8.67± 0.58), respectively.

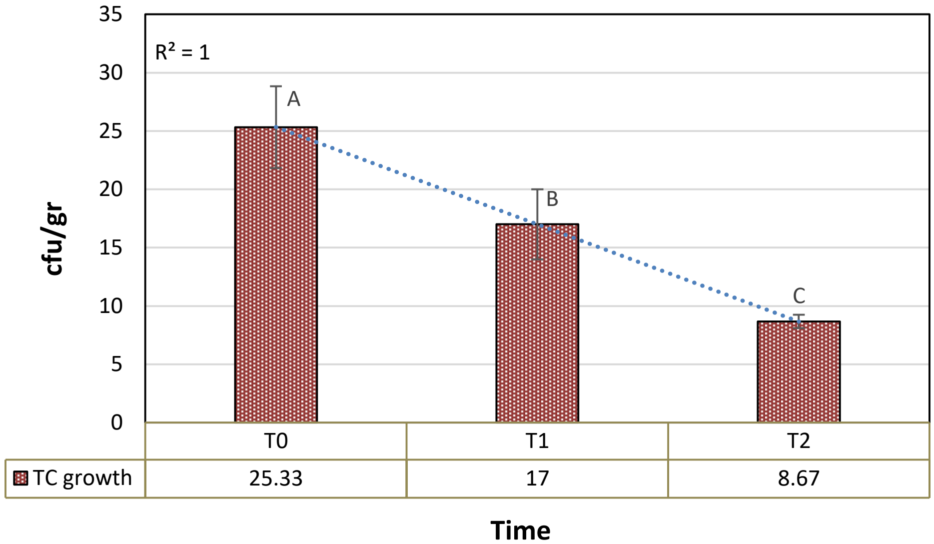


Fig.4.25: Changes on TC count of the TG white- brined cheese sample during storage time. ± Letters (A - D) indicate the statistical differences (at 0.05 level, Fisher test) between different storage periods of TG treated WBC sample.

In general, the decrease in total coliform numbers probably attributed to increase activity of lactic acid bacteria, and pH decline upon time (El Owni and Hamid, 2008).

The coliform results were in accordance with those of Babel (1977); Aly and Galal (2002) who found that coliform numbers of Domiati cheese decreased during storage from ($3.5*10^5$) at day zero to ($1.2*10^5$) at day (120).

Maher et al., (2001); and Ahmed (1985) who found that E-coli counts of smear- ripened cheese, produced from raw milk, decreased during storage time. The decrease could be due to the effect of high storage temperature and high salt level on their growth.

Comparing the total coliform results of the control WBC and TG- WBC, it can be concluded that the TG- WBC total coliform numbers during storage were lower than the control WBC numbers.

Its noteworthy that the decrease in total coliform numbers during storage was higher at TG- WBC than the control WBC. This can be explained by the influence of TG crosslinking, that reduce the bioavailability of some necessary nutrients for microbial growth (Mahmood and Sebo, 2009; Han et al., 2003).

4.2.4.3 Yeast and Mold Growth during Storage Time.

The yeast and mold count were significantly affected by storage period, as they revealed a continuous increase with storage time progress.

Yeast and mold count of the control WBC exhibited a significant increase as storage time increase (Fig.4.26).

Yeast and mold count at (T0) was (113.67±0.58). As the time progressed from (T1 to T2), yeast and mold count was increased from (146.67±5.51, to 180±16.00).

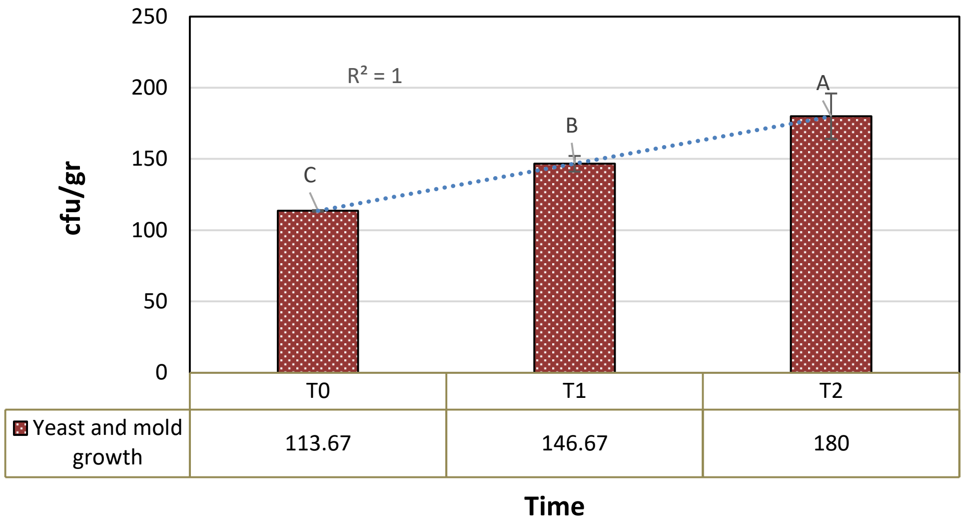


Fig.4.26: Changes on yeast and mold count of the control white- brined cheese sample during storage time. ± Letters (A - D) indicate the statistical differences (at 0.05 level, Fisher test) between different storage periods of control WBC sample.

Yeast and mold results of TG- WBC showed a non-significant increase at the first (15) day of storage, followed by a significant sharp increase at the second (15) day of storage (Fig.4.27).

At (T0), yeast and mold result were (91±2.65), at (T1) the obtained result exhibited a non-significant increase which was (123±8.66), and at (T2) a significant increase marked, the result was (273±63.00).

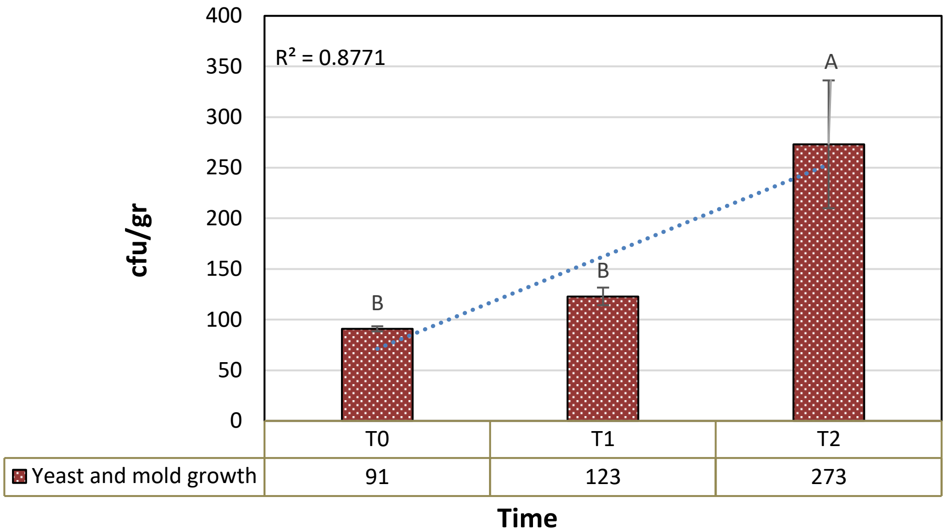


Fig.4.27: Changes on yeast and mold count of the TG white- brined cheese sample during storage time. ± Letters (A - D) indicate the statistical differences (at 0.05 level, Fisher test) between different storage periods of TG treated WBC sample.

Yeast and mold results were in accordance with El Owni and Hamid (2008) who reported that the Sudanese white cheese yeast and mold counts were significantly increased with progress in storage time. Turkoglu et al., (2003) who reported a significant negative correlation between yeast and mold counts and coliform counts due to acid forming ability.

Comparing the increasing counts of yeast and mold in the control WBC and TG- WBC during storage time. The TG- WBC counts exhibited higher increase with time progress than the control counts.

The pattern of change in titratable acidity, pH, and moisture content of WBC upon storage, correlated with yeast and mold growth stimulation. Moisture content is known to be one of the most critical components in promoting mold growth (Block, 1953). TG- WBC showed higher moisture content upon storage time than the control. Thus, beside pH decline, higher moisture will facilitate higher growth.

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CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

Conclusion

In this study the Influence of different pasteurization temperatures combined with different milk TG treatments on the yield and quality attributes of concentrated yogurt (Labneh) and local white- brined cheese (WBC), were presented. The study revealed the following conclusions:

1. TG enzyme crosslinking is strongly dependent on the milk heat treatment.

2. Heat treatment and TG enzyme combination had a significant effect on the concentrated yogurt (Labneh), and local white- brined cheese (WBC) yield percentage.

3. Heat treatment and TG enzyme combination had a significant effect on whey protein incorporation.

4. The optimum combination of pasteurization temperature and TG enzyme ratio, that gave the highest concentrated yogurt yield with lowest whey loss, was (90 C°/ 15 sec, with 3 gm TG/ kg milk).

5. The optimum combination of pasteurization temperature and TG enzyme ratio, that gave the highest local white- brined cheese yield, was (72 C°/ 15 sec, with 3 gm TG/ kg milk).

6. TG crosslinking influenced the chemical attributes of the experimental products. The treated samples exhibited higher pH values, and lower TA values during storage time compared to the control.

7. In concentrated yogurt (Labneh) experiments, TG treated samples showed higher WHC, and lower syneresis during storage period, than the control samples.

8. In local white- brined cheese (WBC) experiments, TG treated samples exhibited higher moisture content and lower DM during time, than untreated samples.

9. TG crosslinking influenced the product microbial growth greatly. It retarded the growth of LAB, which influence the acidification process, and the growth of pathogenic microbes such as total coliform; that occur due to the reduced availability of necessary nutrients needed for microbial growth, as these nutrients cross-linked in the casein network. However, yeast and mold growth enhanced, due to higher moisture retention caused by TG crosslinking in treated samples.

10. Coliform were not detected on fresh Labneh samples, and during storage period which reflects adequate hygienic conditions during product processing.

11. The presence of coliform bacteria along with yeast and mold in local white- brined cheese, indicates substandard hygienic practices during the production process.

12. Microbiological analysis revealed that the hygienic conditions were essential to preserve the product quality throughout their shelf life.

Recommendations

1. Pasteurization temperature is of major concern to produce a safe product, and increase proteins susceptibility to TG crosslinking.

2. TG enzyme is recommended as useful method for improving the yield of concentrated yogurt and white brined cheese.

3. Crosslinking of milk proteins by means of TG appears to be an acceptable alternative instead of addition extra protein or stabilizers.

4. TG had a slight adverse effect on the growth of lactic acid bacteria, which affects their activity, so specific starter culture (for flavor or aroma), may be needed to be selected.

5. In TG treatments, the use of antifungal is necessary to limit yeast and mold growth during storage.

6. Good manufacturing conditions are important for good quality product.

7. In terms of reducing whey loss, we recommended to use the whey as a pickling solution for cheese preservation; due to its positive impact on product quality, and it reduce the moisture loss during storage period because the curd absorb the pickling whey (Dariani et al., 1980).

8. Further studies are needed to highlight the cost of TG enzyme incorporation in concentrated yogurt and white brined cheese.

9. Further studies are needed to investigate the sensorial properties of the treated samples (from the optimum combinations), to develop these products with high savor.

10. Further studies are needed to investigate the rheological properties of the treated samples (from the optimum combinations).

11. Further studies are needed to investigate the enzyme time of addition to increase yield percentage.

12. Further studies are needed to investigate the effect of transglutaminase addition on the fermentation or coagulation time; to take time cost into consideration.

13. The obtained results were established for cow milk, cannot be extrapolated to other milk sources such as buffalo or goat milk.

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APPENDICES

Appendix One Physicochemical tests

1. Concentrated Yogurt Yield and Whey Percentages Measurement

Yield indicates the amount of product that can be obtained, which relates to the efficiency of the manufacturing processes.

1.1. Apparatus

1.1.1. Analytical balance.
1.1.2. Glass beakers (500 ml).

1.2. Procedure

1.2.1. Weigh plain yogurt in clean beaker before the yogurt straining.

1.2.2. After bag straining for partial whey removal, weigh yogurt curd in clean beaker.

1.2.3. Calculate yield percentage.

1.2.4. Collect and weigh the obtained free whey that pooled in the container from the bottom of the strainer.

1.2.5. Calculate whey percentage.

1.3. Calculations

$$Yield\ \% = Yogurt\ curd\ weight(kg) / \ Plain\ yogurt\ weight(kg) * 100\%$$

$$Whey\ \% = Free\ whey\ weight(kg) / \ Plain\ yogurt\ weight(kg) * 100\ \%$$

2. White Cheese Yield Percentage Measurement

2.1. Apparatus

2.1.1. Analytical balance.

2.1.2. Glass beakers (500 ml).

2.2. Procedure

2.2.1. Weigh the input milk quantity of the cheese making.

2.2.2. Weigh the obtained final cheese curd after pressing.

2.2.3. Calculate cheese yield percentage.

2.3. Calculations

$$Cheese\ yield\ \% = Cheese\ weight(kg) / \ Milk\ weight(kg) * 100\%$$

3. Syneresis, and WHC of Concentrated Yogurt (Labneh)

3.1. Apparatus

3.1.1. Centrifuge.

3.1.2. Centrifuge tubes.

3.1.3. Analytical balance.

3.1.4. Glass beakers (250 ml).

3.1.5. Spatula.

3.2. Procedure

3.2.1. Weigh approximately (10 gr) of concentrated yogurt curd sample.

3.2.2. Place the sample in a centrifuge tube using spatula.

3.2.3. Place the sample tube in the centrifuge device, then centrifuge for (10 min) at (2500 rpm) under room temperature.

3.2.4. Weigh the supernatant or the expelled whey in a clean beaker.

3.2.5. Calculate syneresis (%).

3.2.6. Calculate WHC (%).

3.3. Calculations

$$Syneresis\% = Supernatant\ weight\ (gr) / \ Sample\ weight\ (gr) * 100\%$$

$$WHC\% = \frac{Sample\ weight(gr) - Supernatat\ weight(gr)}{Sample\ weight(gr)} * 100$$

4. Moisture and Dry Matter (DM) Contents of White- Brined Cheese

4.1. Apparatus

4.1.1. Moisture analyzer.

4.1.2. Moisture analyzer weighing pan.

4.1.3. Spatula.

4.2. Procedure

4.2.1. Start up the moisture analyzer.

4.2.2. Put (5-10 gr) sample in the weighing pan.

4.2.3. Close the lid to start the drying process.

4.2.4. Drying process ends when the test is over, Where the device will give a signal.

4.2.5. The obtained result reflects the moisture**%** of the tested sample.

4.2.6. Calculate dry matter percentage.

4.3. Calculations

$$Dry\ matter\% = 100 - moisture\%$$

5. Titratable Acidity

Titratable acidity is an approximation of the total acidity in a substance. It determines how much of a base (NaOH) is required to neutralize an acid.

5.1. Apparatus

5.1.1. (0.1 N) sodium hydroxide.
5.1.2. Phenolphthalein indicator.
5.1.3. Analytical balance.
5.1.4. Burette.
5.1.5. Magnetic stir plate.
5.1.6. Magnetic stir bar.
5.1.7. Conical flask (250 ml).
5.1.8. Dropper.
5.1.9. Distilled water.
5.1.10. Graduated cylinder.

5.2. Procedure

5.2.1. Fill the burette with (0.1 N) NaOH.
5.2.2. Weigh (10 gr) sample, for solid and semi solid samples mix with (100 ml) of distilled water in (250 ml) conical flask.
5.2.3. Add approximately (4) drops of phenolphthalein indicator.
5.2.4. Titrate by adding titrant (NaOH) to the sample gradually with continuous mixing on stir plate until the solution color changes to a persistent light pink.
5.2.5. Record the amount of titrant that was needed to neutralize the lactic acid.
5.2.6. Calculate titratable acidity.

5.3. Calculations

$$Titratable\ acidity\% = \frac{Volume\ of\ titrant \text{ x } N \text{ x}90}{Weight\ of\ sample \text{ x } 1000} \text{ x } 100$$

Where, N = normality of titrant; 90 = Equivalent weight for lactic acid.

6. pH Test

6.1. Apparatus

6.1.1. pH meter.

6.1.2. Standard buffer solutions (4 and 7 buffers).

6.1.3. Analytical balance.

6.1.4. Glass beakers (250 ml).

6.1.5. Distilled water.

6.2. Procedure

6.2.1. Weigh sample size of (10 gr), for solid and semi- solid samples mix entirely with (10 ml) distilled water to make a slurry.

6.2.2. Insert pH meter electrode into the sample slurry.

6.2.3. Take pH reading when the value had stabilized (typically < 5min).

Appendix Two Microbiological Tests

1. Food Sampling

1.1. Sterilize forceps, spatulas, and scissors in an autoclave to be used for sampling.
1.2. Use sterile sampling equipment to transfer a representative sample to sterile sample bag under aseptic conditions.
1.3. Identify each sample unit with a properly marked strip of masking tape.
1.4. If the sampling done under aseptic conditions out of the laboratory, transfer the samples to sterile containers under aseptic conditions.
1.5. Deliver samples to the laboratory promptly with the original storage conditions maintained as nearly as possible to be tested.

2. Testing Equipment and Materials

2.1. Work area, level table with ample surface in room that is clean, well-lighted, well-ventilated, and reasonably free of dust.
2.2. Storage space, free of dust and insects and adequate for protection of equipment and supplies
2.3. Petri dishes, glass or plastic (90 mm)
2.4. Dilution tubes (250 ml), with plastic screw caps
2.5. Petri dish containers, adequate for protection
2.6. Circulating water bath, for tempering agar, thermostatically controlled to (45 ± 1C°).
2.7. Incubators, (37 ± 1C°) and (25 ± 1C°).
2.8. Colony counter, with suitable light source and grid plate.
2.9. Anaerobic jar.
2.10. Stomacher.

2.11. Thermometer.

2.12. Micropipette and (1 ml) tips.

3. Culture Media and Reagents

3.1. Peptone water

3.2. Culture media:

3.2.1. Plate count agar culture media to determine total bacterial count.

3.2.2. Violet red bile agar media to determine total coliform count.

3.2.3. MRS agar medium agar medium to determine lactic acid bacteria.

3.2.4. Potato dextrose agar to determine yeast and mold count.

4. Sample Preparation

4.1. Weigh (10 gr) sample under aseptic conditions.

4.2. Add (90 ml) sterile peptone water to make (10^{-1}) dilution.

4.3. Homogenize the sample using stomacher.

4.4. Prepare decimal dilutions if needed, by transferring (1 ml) of (10^{-1}) dilution to (9ml) sterile peptone water (10^{-2}) dilution obtained, transfer (1 ml) of (10^{-2}) to (9ml) sterile peptone water dilution (10^{-3}) obtained, and so on. (in our study (10^{-2}-10^{-5}) dilutions for Labneh samples testing, and (10^{-2}) dilution for white- brined cheese samples).

5. Sample Culturing for Total Bacterial Count

5.1. Prepare culture medium (PCA) according to manufacturer's instructions. Cool to (48C°) before use.

5.2. Prepare, homogenize, and decimally dilute sample as described in the previous sections.

5.3. Transfer (1 ml) of the tested dilution to petri dishes.

5.4. Pour (20-25 ml) of the culture medium tempered to (48C°) into plates.

5.5. Swirl plates to mix, and let solidify.

5.6. Invert solidified plates and incubate at (37C°) for (48 hr.).

5.7. Count the colonies forming units (cfu).

6. Sample Culturing for Total Coliform Count

6.1. Prepare culture medium (VRBA) according to manufacturer's instructions. Cool to (48C°) before use.

6.2. Prepare, homogenize, and decimally dilute sample as described in the previous sections.

6.3. Transfer (1 ml) of the tested dilution to petri dishes.

6.4. Pour (20-25 ml) of the culture medium tempered to (48C°) into plates.

6.5. Swirl plates to mix, and let solidify.

6.6. Invert solidified plates and incubate at (37C°) for (48 ± 2 hr.).

Count purple-red colonies that are (0.5 mm) or larger in diameter and surrounded by zone of precipitated bile acids.

7. Sample Culturing for Yeast and Mold Count

7.1. Prepare culture medium (PDA) according to manufacturer's instructions. Cool to (48C°) before use.

7.2. Prepare, homogenize, and decimally dilute sample as described in the previous sections.

7.3. Transfer (1 ml) of the tested dilution to petri dishes.

7.4. Pour (20-25 ml) of the culture medium tempered to (48C°) into plates.

7.5. Swirl plates to mix, and let solidify.

7.6. Incubate at (25-28 C°) for (72 hr.).

7.7. Count the colonies forming units (cfu).

8. Sample Culturing for Lactic Acid Bacteria

8.1. Prepare culture medium (MRS) according to manufacturer's instructions. Cool to (48C°) before use.

8.2. Prepare, homogenize, and decimally dilute sample as described in the previous sections.

8.3. Transfer (1 ml) of the tested dilution to petri dishes.

8.4. Pour (10 ml) of the culture medium tempered to (48C°) into plates, swirl plates to mix, and let solidify.

8.5. overlay with (8-10 ml) of melted, cooled MRS. Swirl plates to mix and let solidify.

8.6. Invert solidified plates and place them in anaerobic jar.

8.7. Incubate the samples jar at (37C°) for (48 ± 2 hr.).

8.8. Count the colonies forming units (cfu).