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Assessment of Milk Quality and Antibiotic Residues Detection in Milk Samples from Palestinian Market

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Dedication

First of all, my greatest gratitude to Allah

To my patient and supportive husband Adnan

To my lovely sons Mohammed, Ady and Laith

To my mother and father

To my sisters and brothers, especially lovely Saleem and his family

for their encouragement and support during this study.

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Last but not least, I am grateful and indebted to my precious family, my husband and sons, and for all my friends.

الإقرار

أنا الموقع أدناه مقدم الرسالة التي تحمل عنوان:

Assessment of Milk Quality and Antibiotic residues Detection in Milk Samples from Palestinian Market

أقر بأن ما اشتملت عليه هذه الرسالة، إنما هي نتاج جهدي الخاص، باستثناء ما تمت الإشارة إليه حيثما ورد، وأن هذه الرسالة ككل، أو أي جزء منها لم يقدم لنيل أي درجة علمية، أو بحث علمي أو بحثي لدى أي مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provided in this Thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's name: **اسم الطالب:**

Signature: **التوقيع:**

Date: **التاريخ:**

LIST OF ABBREVIATION

ADI	Acceptable Daily Intake
AMRs	Antibiotic Maximum Residues
AR	Antibiotic Residue
EMA	European Medicines Agency
EU	European Union
FAO	Food and Agriculture Organization United Nation
FP	Freezing Point
HPLC	High-Performance Liquid Chromatography
Kg	Kilogram
LC	Liquid Chromatography
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
Max	Maximum
Min	Minimum
MoA	Minister of Agriculture
MRL	Maximum Residue Limit
PCBS	Palestinian Central Bureau of Statistics (PCBS)
pH	Hydrogen ion concentrations
SCAMAC	Standing Committee on Analytical Methods for Additives and Contaminants
SNF	Solid not Fat
TS	Total Solids
UHT	Ultra-Heat Treatment
US	United State
VAPRO	Vapor Pressure Osmometer
WHO	World Health Organization

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Abstract

This study was conducted to evaluate the quality of milk in the Palestinian market during a period of 8 months, from June 2017 to January 2018. A total of 118 milk samples, of which 79 pasteurized, 14 raw and 25 (UHT) were collected randomly from the market, using the production date as the collection criteria. The samples were screened for antimicrobial residues using Delvotest SP-NT, and their osmolality was measured using Vapor Pressure Osmometer (VAPRO). In addition, the pasteurized and UHT milk samples were evaluated for physicochemical properties (fat, solids not fat; SNF; protein, lactose, ash, freezing point and pH), using (Ultrasonic Milk Analyzer) and pH meter. The results of the antibiotic screening test SP-NT showed that (36%) raw milk samples contained antibiotic residues above MRL. Meanwhile, the pasteurized milk samples (27%) were contaminated with antibiotic residues above MRL. None of 25 UHT milk samples were contaminated with antibiotic residue.

The average content of fat and solids in pasteurized milk samples was higher during winter (3.38%, 0.65%, respectively). The Freezing point and pH average were higher during summer (-0.5048°C, 6.78, respectively). Moreover, the average content of SNF, Protein and lactose

were higher in autumn (8.04%, 2.96%, and 4.41%, respectively). The average content of (fat, SNF, ash, protein, lactose) and FP of pasteurized milk samples revealed non-significant differences (P>0.05) between seasons. Meanwhile, the average pH showed significant differences (P<0.05) between seasons.

The results showed that the average content of fat and protein in UHT milk samples were higher in spring (3.28%, 3.18%, respectively), the average content of solids and freezing point were higher in summer (0.65%, -0.5087°C, respectively). Moreover, the average of SNF and pH were higher in autumn (8.01%, 6.74, respectively). The Physiochemical properties average (fat, solids not fat; SNF; protein, lactose, ash, freezing point and pH) of UHT milk samples revealed non-significant differences (P>0.05) between seasons.

The osmolality average of raw milk samples, pasteurized and UHT was (309±37.86, 273±24.08, 271.76±7.46 mOsmol/kg, respectively). Moreover, the osmolality average of milk samples revealed significant differences (P<0.05) between raw and heat-treated milk samples.

The present study concluded that physiochemical properties and the osmolality of milk were affected by seasonal variation. Also, the study showed that the percentage of contaminant raw milk samples by antibiotic residue above MRLs is higher compared to that heat-treated milk samples.

CHAPTER ONE
Introduction

Chapter One

1.1. INTRODUCTION

Milk contains an optimal balance of proteins, fats, carbohydrates, vitamins (A, B2, B12 and D), calcium, phosphorus, potassium and other minerals providing a range of benefits for growth, immunity, and development for the calves and also to humans (Padol *et al*., 2015; Mahmoudi *et al*., 2014). Cow's milk is one of the most consumed foods by all populations from newborn to the elderly. Thus milk and milk products intended for human consumption must be safe, without microbiological, physical or chemical contaminants like toxic metals, mycotoxins, radionuclides, pesticide and veterinary drug residues. Milk must not be adultered by water addition (Trombete *et al,* 2014; Zanella *et al.,* 2010).

Cow's milk contains approximately 87% water. All components vary within the same species, and also from one dairy to the other, depending on cows' race, age, the period of lactation, diet and other environmental factors (Büttel *et al.,* 2008; Guetouache *et al.,* 2014). The osmolality of milk is a significant value owing to its being highly preserved even when collected from a larger population of animals. Therefore, osmolality can be used in milk quality control (Büttel *et al.,* 2008).

Antibiotics are widely used in food animal production. They are used for treating the bacterial infections at large dosage, and as feed additive at a low dosage for a long period in order to prevent diseases, promote growth and increase feed efficiency (Abbasi *et al.,* 2011; Aalipour *et al.,* 2013;

Darwish *et al.,* 2013). The extensive use of these antimicrobials, insufficient withdrawal period and lack of good veterinary practice cause the presence of residues in milk. Illegal or off-label use of drugs and incorrect dosage levels or dosing schedule enlarge the problem. (Al-Zuhair, 2012; Kebede *et al.*, 2014; Padol *et al.*, 2015).

Antibiotic residues in milk are a potential hazard for public health. They may cause allergic reactions (B-Lactams), development of bacterial resistance which can transfer to human through the food chain and environment, making treatment of human infections difficult (Darwish *et al.*, 2013), carcinogenicity (Sulphamethazine, Oxytetracycline, Furazolidone), mutagenicity, immunopathological effects, autoimmunity, nephropathy (Gentamicin), hepatotoxicity, reproductive disorders, bone marrow toxicity (Chloramphenicol), (Nampoothiri *et al.*, 2014; Darwish *et al.*, 2013; Padol *et al.*, 2015; Nisha, 2008; Abdelmoaty, 2015). Antibiotic residues interfere with the balance of intestinal micro-flora and cause delay or failure in dairy fermentation like cheese and yogurt causing economic losses (Aalipour *et al.*, 2013; Kaya *et al.*, 2010; Movassagh, 2011).

About 50-80% of antibiotics are excreted through urine and feces. Using manure as fertilizer can contaminate soil, surface, and groundwater which results in contamination of food and drinking water (Rasouli *et al.*, 2015; Gothwal *et al.*, 2014).

The degree of milk contamination with antibiotic residues varies from country to another, depending on the level of education, legislation, and food inspection (Kaya, 2010).

To ensure human food safety, worldwide regulatory authorities such as world health organization (WHO) (1999) and the food and agriculture organization (FAO) (2008) have set standards for acceptable daily intake (ADI) and maximum residue levels (MRLs) for several veterinary drugs in food. (Mesragi *et al*., 2011; Oluwafemi *et al.,* 2018).

1.2. OBJECTIVES

The objective of the present study is to assess the quality of raw, pasteurized and Ultra Heat Treated (UHT) milk sold in the Palestinian market through:

1- Screening milk samples for antibiotic residues.

2- Determining the osmolality of milk samples.

3- Determining the milk's physiochemical properties (fat, protein, SNF, solids, lactose, FP and pH).

4- Determining the effect of the season's variation on the quality of milk.

1.3. RESEARCH PROBLEM

Understanding the quality and safety of milk and milk products sold in the local Palestinian market is very crucial. No studies have been done and

there is a need to screen marketed milk from different sources. This study will cover the presence of antibiotic residues, the presence of added water and other physiochemical properties of milk in relation to season so as to assess the quality and safety of milk.

Milk producers and farmers will be assessed indirectly for understanding the importance of hygiene practices during cows breeding, milking, and processing of milk. In addition, it will help regulatory authorities and milk industry in the formulation of control strategies on the use of antibiotics as a veterinary drug, and also understand the environmental variations and its effect on physicochemical properties of milk.

CHAPTER TWO

Literature Review

Chapter Two

2. LITERATURE REVIEW

2.1. PHYSIOCHEMICAL PROPERTIES OF MILK

Milk is the normal mammary secretion of milking animals obtained from one or more animals without either addition to it or extraction from it, intended for consumption as liquid milk or for further processing (FAO Codex, 1999). Milk contains all of the nutrients necessary for survival and the initial growth of mammalian neonates. The nutrients in milk include sources of energy (lipids and carbohydrates), proteins to provide amino acids, vitamins and minerals for electrolytes, and water (Husvéth, 2011). The major cow milk producers worldwide are The European Union (148.1 million kg3), The United States of America (85.9 million kg3), India (45.1 million kg3), and Russia (32.3 million kg3). Table 1 and figure 1 show the proximate composition of whole bovine milk from different countries (Schönfeldt *et al.*, 2010).

Table (1): Proximate composition of whole bovine milk from different countries.

Whole bovine milk/100g	South Africa	USA	UK	Denmark	Australia New Zealand
Water	88.0	88.3	87.8	87.8	87.5
Energy by KJ	260	252	275	269	278
Protein	3.25	3.22	3.20	3.40	3.30
Lactose	4.80	5.26	4.80	4.64	4.70
Fat	3.43	3.25	3.90	3.50	4.00
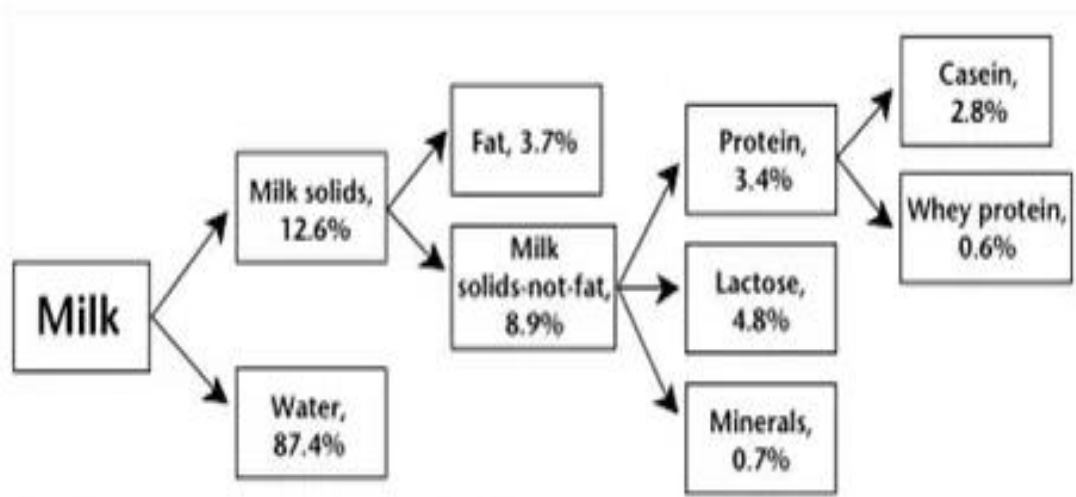
Figure (1): Milk composition

2.2. MILK PRODUCTION IN PALESTINE

Livestock in Palestine is not only an economic or income generation activity but also a distinctive trait, cultural and tradition of the Palestinian people. There were 33,980 cows raised in the Palestinian territory, including 25,612 cows in the West Bank and 8,368 in the Gaza Strip. Of which, 32% were local cows (Baladi and mixed breeds) and 68% other strains (Holstein, Friesian, and hybrid cows) (PCBS, 2013). 87.6% of cows are raised through intensive breeding, 12.2% through semi-intensive breeding, and 0.2% by an unknown type of breeding (PCBS, 2010). Local demand for milk and dairy products was estimated at 204 million litter a year. 89% of local market needs are produced locally and the remaining gap is covered by imports, approximately 17,416 million litter of liquid milk equivalent are imported a year. Table 2 shows the imported and exported amounts of milk in thousands of dollars (PCBS, 2017).

Table (2): In 2015, the imported and exported amounts of milk in thousands of dollars.

Country	Imported	Exported
Jordan	0	186
Egypt	53	0
France	2	0
Israel	5,772	315

The consumption amount of fresh and pasteurized milk was estimated in 2015 at 33,031,993 liters. Industrial production includes 10 processing plants that process 155,000 tons of raw cow's milk per year (MoA, 2014).

The average quantity of Palestinian household monthly consumption of milk (Table 3) was 4.4 liters, 4.7 liters of yogurt and 1.5 kg of different types of cheese (PCBS, 2010; PCBS, 2017).

Table (3): The average quantity of monthly consumed milk in liters by Palestinian household, 2006-2011.

Year	West-Bank	Gaza	Average
2006	5.475	2.489	4.478
2007	5.005	1.833	3.982
2009	4.852	1.270	3.730
2010	4.894	1.979	3.977
2011	4.807	0.884	3.527

2.2.1 FAT

Most of the lipids in milk are in the form of triglycerides, and these are the primary source of dietary energy in milk. Triglycerides are composed of three fatty acids and glycerol (Husvéth, 2011). Milk fat has

the most complex fatty acid composition of the edible fats. Over 400 individual fatty acids have been identified in milk fat (Mansson, 2008). However, approximately 15 to 20 fatty acids make up 90% of the milk fat. Milk fat globules have an average diameter of less than 0.1μm to approximately 18 μm and consist of a triglyceride core surrounded by a natural biological membrane (Ghalib, 2014).

Milk fat can be degraded by enzyme action, exposure to light, and oxidation. Enzymes that degrade fat are called lipases, and the process is called lipolysis. Usually, the action of lipase causes undesirable rancid flavors in milk. Pasteurization inactivates lipases and increases the shelf life of milk. Milk fat is fully melted at (40°C). Typical high-temperature short time (HTST) pasteurization conditions do not affect the functional and nutritional properties of milk fat. Higher heat treatments may stimulate oxidation reactions and cause fat deterioration and off-flavors such as Ultra High Temperature (UHT). Milk fat acts as a solvent for the fat-soluble vitamins A, D, E and K and also supplies essential fatty acids (linoleic, linolenic and arachidonic) (Cortes, 2011). Milk fats are the most variable compounds, being under the influence of species, breed, lactation stage, feeding, season, and health condition (Grădinaru, *et al.,* 2015; Linn, 1988). A study conducted by Galib to investigate raw cow milk composition showed that the average fat content was found as 5.03±0.04% during the winter season, and 4.30 ±0.6% during summer season (Ghalib, 2014), In Kenya, Kabui found that the average of fat content was 3.8% (Kabui, 2012). In Iran, Nateghi *et al* reported that fat content was 3.8% in summer

and 3.41% in winter (Nateghi *et al.,* 2014). Abd Elrahman reported that the average fat content was 4.14% in a study conducted in Sudan.

2.2.2 PROTEIN

Proteins are chains of amino acid molecules connected by peptide bonds. There are 20 different amino acids in proteins. The content and sequence of amino acids in a protein affect its properties. Some proteins contain substances other than amino acids, e.g. lipoproteins. The dominant class of protein in milk called casein. Bovine milk contains about 3.5% protein, casein constitutes about 80% and it is easily separated from milk, either by acid precipitation or by adding rennin. Whey proteins constitute about 20% and exist as individual units dissolved in the water phase of milk. The major whey proteins are beta-lactoglobulin and alpha-lactalbumin. (Cortes, 2011; Linn, 1988). Mirzadeh found that milk protein was 3.3±0.22% in a study conducted in Iran (Mirzadeh *et al*., 2010). In another study conducted in Sudan by Elsheikh, milk protein was found as 3.58±0.33% (Elsheikh *et al*., 2015). In the world, Friesian and Holstein cow milk averagely contains 3.3% protein (Mirzadeh *et al*., 2010). Abd Elrahman studied the physicochemical properties of bovine milk and showed that milk protein average was 3.47±0.012 (Abd Elrahman, *et al*., 2009). Kabui found that the average of milk protein was as 3.1% (Kabui, 2012). Natighi found that milk protein amounts in summer and winter were 3.71% and 3.01%, respectively (Nateghi *et al.,* 2014). These variations are

results of different animal feeding in summer and winter, age and health, and the environment (Abd Elrahman, *et al*., 2009; Nateghi *et al.,* 2014).

2.2.3 SOLIDS NOT FAT (SNF)

Solid not fat contains the protein, the minerals, and the milk-sugar which collectively make milk such valuable and palatable food. Liquid milk for human consumption must contain at least 8.5 percent, solids-not-fat (Snook, 1960). Kabui, 2012 found the average of SNF in milk was 8.2%. In another study, Thomas found that the solid not fat percentage in milk was 8.3% (Thomas *et al*., 2015). Galib found that the average content of solids not fat of milk samples was 11.52±2.3% during the winter season. Similarly, the mean value of SNF was 11.21±0.6 % in summer (Galib, 2014).

2.2.4 LACTOSE

Lactose (Figure 1) is the major carbohydrate fraction in milk. It is made up of two sugars, glucose, and galactose. The average lactose content of milk varies between 4.7% and 4.9%. Mastitis reduces lactose secretion. Lactose is dissolved in the serum (whey) phase of fluid milk. The lactose content of milk in summer and winter being reported by Nategi as 4.61% and 4.58% respectively (Nateghi *et al.,* 2014). Ghalib showed that the average lactose content in milk samples was 4.62 ±0.2 in winter and 4.72±0.2 in summer.
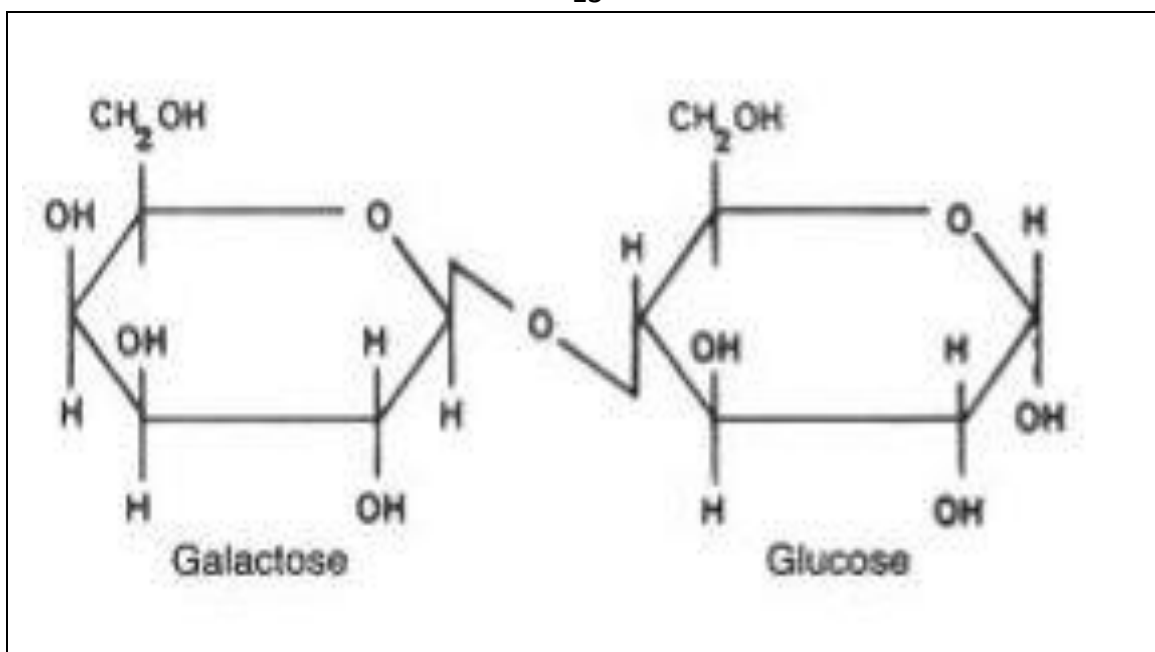
Figure (2): Structure of lactose molecule

2.2.5 HYDROGEN ION CONCENTRATION (PH)

The pH or the hydrogen ion concentration of milk gives a measure of the acidity of milk. In normal cow milk, the pH ranges from 6.6 to 6.8. The pH value can be lower than 6.6. The pH of milk is mainly attributed to the phosphates, citrates and carbon dioxide present in milk. The pH value can be greater than 6.8 mainly due to mastitis (Kabui, 2012). The pH of milk is more dependent on temperature than that of buffers, such as phosphate; since milk is a complex buffer system and variation in temperature causes many changes. Differences in pH and buffering between individual lots of fresh milk reflect compositional variation (Gakkhar *et al.*, 2015)

Milk pH can be determined directly or indirectly. Direct measurement is through the use of indicator dyes, titratable acidity or use of pH meters. Indirect measurement is done through the clot on boiling and

alcohol tests (Kabui, 2012). Kabui found an average milk pH of 6.63 in a study conducted in Kenya. In a study conducted by Abd Elrahman *et al* the mean of milk pH was 7.02 (Abd Elrahman *et al.,* 2009).

2.2.6 FREEZING POINT

The freezing point of milk is the minimum temperature at which the milk flow becomes null, and the milk viscosity becomes maximum. It is a transition point of liquid-solid phases (Bouisf *et al.,* 2018).

The freezing point of cow's milk has long been recognized as one of its most constant values (Watrous *et al.,* 1975) and it is an indirect measure of the osmotic pressure. Depression of the milk freezing point was often related to an increase in protein and solids content. The freezing point of milk is slightly lower than that of water, because of the presence of dissolved solids. The maximum limits of the freezing point of both raw and heat-treated drinking milk were ≤ –0.520°C according to EU regulations (Buttel *et al.,* 2008). A total of 295 samples of heat-treated drinking milk was examined by Navratelova over a period of one year, 145 of them were pasteurized milk and 150 UHT. The average freezing point was found as -0.516°C, -0.514°C, respectively. (Navratelova *et al.,* 2006). Bouisf *et al* showed that the mean of milk FP was -0,521°C. (Bouisf *et al.,* 2018). Abd Elrahman *et al* found that the average of raw and pasteurized milk FP was respectively -0.520°C, -0.447°C (Abd Elrahman *et al.,* 2009).

2.2.7 MILK SALTS (ASH)

Milk salts are mainly chlorides, phosphates, and citrates of sodium, calcium and magnesium. Although salts comprise less than 1 % of the milk, they influence its rate of coagulation and other functional properties. Calcium, magnesium, phosphorous and citrate are distributed between the soluble and colloidal phases.

Their equilibria are altered by heating, cooling and by a change in PH, milk also contains trace elements that come to the milk from feeds or milking equipment such as copper, iron, nickel and zinc (Cortes, 2011). The average ash content varied from 0.74% for Holsteins to 0.83% percent for Jerseys. The highest calcium and phosphorus contents in milk were reported for Jerseys (Linn, 1988). Elbagermi *et al*., 2014 reported that the average content of ash in cow milk was 0.63% in a study conducted in Libya (Elbagermi *et al.,* 2014).

2.2.8 MILK OSMOLALITY

Osmolality is the number of solute molecules that are dissolved in 1 kg of solvent (Buttel *et al*., 2008). Osmolality is the concentration of solute particles in a solution (James, 2018). The osmolality of milk is a significant value owing to its being highly preserved even when collected from a larger population of cows. Osmolality has been used for quality control of milk because the water content is a preserved parameter changed by external factors. When milk processing takes place water may be mixed

into the milk. This can occur intentionally by adding water or accidentally through water presence in milking machine pipes. The osmolality value is 277 mOsmol/kg corresponds to -0.515℃ as recommended by the German Food Regulations for Milk (Buttel *et al.,* 2008). Milk has an osmolality of 280–290 mosmol/kg (James, 2018). In Germany, the osmolality of 12 heat-treated milk samples was tested by Buttel et al., and the average was found as 271 mOsmol/Kg.

2.3. EFFECT OF HEAT TREATMENT ON MILK COMPOSITION

Pasteurization does not reduce the fat content of milk. Pasteurization has little effect on the nutritional value of milk. There is some loss of vitamin C and B group vitamins, but this is insignificant (FOA, 2013). The process kills many fermentative organisms as well as pathogens. Pasteurized and ultra-high treatment milk keeping protein and lactose content similar to raw milk. (Pestana, 2015).

2.4. ANTIBIOTICS

Antibiotics are organic chemical compounds that kill (Bactericidal) or inhibit the growth (Bacteriostatic) of microorganisms but cause little or no damage to the host (Albayoumi, 2015; Metzeler, 2018). Antibiotics are medicines used to prevent and treat bacterial infections (WHO, 2018). They are naturally produced by microorganisms such as fungi (e.g. penicillin) and bacteria (e.g. tetracycline) or can be semi-synthetically produced (e.g. amoxicillin) or totally synthetically produced (e.g.

sulfonamides) (Albayoumi, 2015). Antibiotic compounds are differentiated as antibacterial, antifungals and antivirals to reflect the group of microorganisms they affect (Etebu, 2016). Table 4 shows the chemical structure of certain antibiotics among β-lactams, Quinolones, Nitroimidazoles, Quinoxalines, Aminoglycosides, Macrolides, Phenicols, Phosphoglycolipids, Lincosamides, Nitrofuran, Ionophores, Sulfonamides, Tetracyclines and Polypeptides (Kebede et al, 2014).

Table (4): Antibiotics classification according to chemical structure.

Group	Internal group	Representative with practical importance
Carbohydrate antibiotics	1.Aminoglycosideantibiotics 2.Other(N- and C-) glycosides	Streptomycin, Neomycin
Macro cyclic lactone (lactam) antibiotics	1.Macrolide antibiotics	Erythromycin
	2.Polyeneantibiotics 3.Macrolactam antibiotics	Amphotericin
Quinone and similar antibiotics		Oligomycin
Amino acid Peptide antibiotics		Tetracyclines Penicillins, Cephalosporins, Bacitracin, Polymyxins
Nitrogen-containing Heterocyclic antibiotics		
Oxygen-containing Heterocyclic antibiotics	1.Non-condensed(single) heterocycles	No practical importance
	2.Condensed (fused) heterocycles	
	1.Furan derivatives	No practical imporance
	2.Pyran derivatives	
Alicyclic antibiotics	1.Cycloalkane derivatives	
	2.Small terpenes	Streptovitacins
	3.Oligoterpene antibiotics	
Aromatic antibiotics	1.Benzene compounds	Chloramphenicol
	2.Condensedaromatic comp.	Grisefulvin
	3.Non-benzene aromatic comp.	Novobiocin
Aliphatic antibiotics	1.Alkane derivatives	Varitin
	2.Aliphatic carbocyclic acid derivatives	

Antibiotics have been used in the dairy industry for more than five decades. The main uses of antibiotics in the dairy industry include:

1. Therapeutic: For disease treatment. The infected animals receive a course of antibiotics in high doses for a short period of time.

2. Prophylactic: For disease prevention in sub-therapeutic doses of antibiotics for a group of healthy animals via feed or drinking water.

3. Disease control: for a group of animals when some of them are sick.

4. Growth Promoters: To increase growth-rate and productivity (Sahu *et al.,* 2014; Hahn *et al.,* 2016; Barton, 2000).

In North America and Europe, it is estimated that about 50% of all antimicrobial production is used in food-producing animals and poultry (WHO, 2001). Van Boeckel *et al.*, 2015 estimated that global consumption of antibiotics in agriculture will increase by 67 percent from 2010 to 2030, and the consumption of antibiotics amongst the BRICS will increase by 99 percent in that same time period (O'Neill, 2015).

2.4.1 ANTIBIOTIC RESIDUES IN MILK

Antibiotic residues are small amounts of drugs or their active metabolites, which remain in milk after treating the cows (Aytenfsu *et al.*, 2016). Antibiotic residues in milk are unacceptable for two reasons. Firstly, there is a potential human health hazard and secondly, antibiotic residues can interfere with the manufacturing process inhibiting yogurt and cheese starter cultures (Edmondson, 2002). The occurrence of antibiotic residues in milk is strongly associated with certain variables such as milk production

rate at the time of treatment, the type and amount of antibiotic used, the type of vehicle used in antibiotic formulations, and the disease state of the animal (Rasoli *et al.*, 2014). Intermammary infusion of antibiotic for treating mastitis is the most common reason for antibiotic residues in milk (92%) followed by injections (6%), and other causes (2%) (Ghowdhary *et al.*, 2015).

A withdrawal period is established to safeguard humans from exposure to antibiotic residues. The withdrawal time is the time required for the residue of toxicological concern to reach safe concentration as defined by tolerance. It is the interval from the time an animal is removed from medication until the permitted time of milking (Nisha, 2008).

According to the results of the technical report prepared for milk hygiene, by World Health Organization and Joint Expert Committee on Food Additives (JECFA), the rate of contamination of milk and dairy products with antibiotic residues in developed countries such as USA, Australia, UK, and Scotch land was 7-10% until 1969, after that year, the rate of contamination of the same products decreased to 0.5% in the USA, 2.1% in Australia, 1.5% in the UK and 3.4% in Scotland due to the precautions are taken after the given date. The same report indicates that in underdeveloped and developing countries the contamination for milk and dairy products might be higher (Kaya, 2010).

In the USA, a study conducted by the Food and Drug Administration (FDA) found that from 1912 samples, 15 (0.79%) was positive for antibiotic residue (FDA, 2015).

In Croatia, from a total of 1259 samples tested for antibiotic residues over three years period, 36 was positive (Bilandzic *et al*., 2011). In Brazil, a study conducted by Brado *et al*., to investigate the presence of tetracycline's in pasteurized milk showed that 3 (3%) of 100 samples were positive (Prado *et al*., 2015). In another study by Fonseca *et al*., who reported from 100 UHT milk samples, 4 (4%) was positive (Fonseca *et al*., 2009). Oleviera *et al*., showed that none of 50 samples of raw milk and 20 pasteurized milk contain antibiotic residue (Oleviera *et al*., 2012).

Reasons for bulk milk contamination by antibiotic residues: (Edmondson, 2002; Galib, 2014).

- Milk from treated cow are accidentally routed into the milking tank
- An antibiotic-treated dry cow unintentionally milked.
- The same milking unit was used to milk an antibiotic-treated cow before milking untreated cows. The milking unit was not cleaned and sanitized between uses.
- Lactating cows were purchased and the new owner was unaware of recent antibiotics prior to sale.
- Equipment used to milk treated cows handled carelessly, for example, vacuum from the milk pipeline was used to operate dump milk buckets.

- All antibiotic-treated dry cows were milk last, but the milk line was not diverted from the bulk tank.

- Medicated feed was accidentally mixed into lactating cow feed.

- Cows drank from medicated footbath.

- One-quarter of a cow was treated for mastitis and withheld from the bulk tank. However, milk from the other three quarters was not withheld and was permitted to enter the pipeline.

- Use of dry cow therapy to treat lactating cows.

There are many natural medicines that are topical and cause little risk for contamination of milk or meat. A few of these "natural treatments" have the potential to be effective in treating mastitis by intermammary infusion. Natural antimicrobials such as caprylic acid, eugenol, trans-cinnamaldehyde, carvacrol, and thymol have been found to be inhibitory, in vitro, towards an array of pathogenic microorganisms and may be potential candidates for effective on antibiotic treatments for mastitis (O'Donnell, 2011).

2.4.2 ANTIBIOTIC RESISTANCE

Antimicrobial resistance is the ability of a microorganism (like bacteria viruses and some parasites) to stop an antimicrobial (such as antibiotics, antivirals, and antimalarial) from working against it. As a result, standard treatment becomes ineffective, infections persist and may

spread to others (WHO, 2018). Antibiotic resistance occurs naturally, but overuse and misuse of antibiotics whether in humans or in animals accelerate the process. O'Neill reported that of 139 academic studies affiliated to universities that address the issue of antibiotic in agriculture, only 7 (5%) argued that there was no link between antibiotic consumption in animals and resistance in humans, while 100 (72%) found evidence of a link (O'Neill, 2015). Antibiotic-resistant bacteria have become a major global public health (Hahn *et al.,* 2016; WHO, 2018), food security and development concern today (WHO, 2018).

Antimicrobial-resistant infections in food-producing animals may have major financial implications for both farmers and consumers (WHO, 2013). Resistant bacteria could transfer to human potential through direct contact with an animal, from consumption of undercooked or unpasteurized animal products, or via the spread of resistant bacteria into environmental reservoirs, which may then transmit resistance genes to human bacteria, or come into contact with humans directly (O'Neill, 2015). For example, a clone of *Salmonella typhimurium* DT104 that has become prevalent in many countries including the UK, Germany and the USA, is resistant to commonly used antibiotics including ampicillin, tetracycline, streptomycin, chloramphenicol and Sulphonamides. Multi-drug resistance has been noted in other *Salmonella* spp (WHO, 2001). The introduction of fluoroquinolone use in poultry has been associated with a dramatic rise in the prevalence of fluoroquinolone-resistant *Campylobacter jejuni* isolated in live poultry, poultry meat and infected humans (WHO, 2001).

Elmanama and Abdelatif, (2012) conducted a study to investigate the antimicrobial resistance for enteric pathogens isolated from acute gastroenteritis patients in the Gaza strip. The study showed that diarrhea was more frequent among peoples living in houses rearing poultry and pigeons. They isolated *Salmonella, Campylobacter coli/jejuni, Aeromonas hydrophilia, Shigella boydii* and *Yersinia enterocolytica*. All isolates were resistant for more than one antimicrobials especially *Campylobacter coli/jejuni*.

Liu et al., (2015) examined areas in China where colistin is routinely given to pigs and they found colistin-resistant *E. coli* in more than 20% of animals and in 15% of raw meat samples, these bacteria all had colistin resistance that could easily be transferred between different bacteria, they also found that about 1% of hospital patients sampled were infected by *E. coli* or *Klebsiella* bacteria that had the same piece of DNA, making them resistant to colistin too (O'Neill, 2015).

In many studies conducted from 2002 to 2013, among private and government hospitals in India, a high level of resistance against common antibiotics was found in several bacteria known to cause common and severe infections (Sahu *et al*., 2014).

2.4.3 ANTIBIOTIC RESISTANCE MECHANISM

Bacteria have genetic plasticity that allows them to respond to environmental threats, including the presence of antibiotic molecules.

Bacteria use two major genetic strategies to adapt to the antibiotic "attack", first, mutations in gene(s) often associated with the mechanism of action of the compound, and second, acquisition of foreign DNA coding for resistance determinants through horizontal gene transfer.

Antibiotic resistance mechanisms can be classified according to the biochemical route involved in resistance, as follows:

1- Modifications of the antimicrobial molecule.

2- Prevention to reach the antibiotic target (by decreasing penetration or actively extruding the antimicrobial compound)

3- Changes and/or bypass of target sites.

4- Resistance due to global cell adaptive processes (Munita *et al.*, 2016).

To prevent and control the spread of antibiotic resistance, the WHO recommended the agriculture sector:

- Only give antibiotics to animals under veterinary supervision.

- Not use antibiotics for growth promotion or to prevent diseases in healthy animals.

- Vaccinate animals to reduce the need for antibiotics and use alternatives to antibiotics when available.

- Promote and apply good practices at all steps of production and processing of foods from animal and plant sources.

- Improve biosecurity on farms and prevent infections through improved hygiene and animal welfare (WHO, 2018).

2.4.4 ADVERSE EFFECT OF ANTIBIOTIC

Antibiotics have the following adverse effects:

1. Allergic or toxic reactions to residues.

2. Chronic toxic effects occurring with sub dosage of antimicrobials for prophylactic or as growth promotion.

3. Development of antimicrobial-resistant bacteria in treated animals. These bacteria might then cause difficult-to-treat human infections.

4. Disturbance of normal human microflora in the intestine, which acts as a barrier to foreign pathogenic bacteria. Antibiotics might selectively kill some important species and reduce their total number (Etebu *et al.,* 2016; Albayoumi, 2015; Jumaa *et al.,* 2015).

2.4.5 MAXIMUM RESIDUE LIMITS

The maximum residue limits (MRL) is the maximum allowed concentration of residue in a food product obtained from an animal that has received a veterinary medicine or that has been exposed to a biocidal product for use in animal husbandry (EMA, 2018). MRLs for Beta-lactams

(Table 5) and Tetracycline (Table 6) have been set by the European Union for food producing animals and carry out control programs and monitoring for drug residues in food to protect public health and avoid economic loss (Kebede *et al.,* 2014).

Table (5): Maximum residue limits (MRL) for beta-lactams.

Antibiotics	MRL(ppb)
Penicillin G	4
Ampicillin	4
Amoxycillin	4
Cloxacillin	30
Dicloxacillin	30
Oxacillin	30

Table (6): Maximum residue limits (MRLs) for tetracycline.

Antibiotics	MRL(ppb)
Tetracycline	100
Chlorotetracycline	100
Oxytetracycline	100
Doxycycline	100

2.4.6 DETECTION OF ANTIBIOTIC RESIDUES IN MILK

Different kinds of analytical methods were developed to determine antibiotic residues in milk such as microbiological, chromatographic, immunochemical, receptor and enzyme-based tests (Trombete *et al*., 2014; Padol *et al*., 2015; Kebede *et al*., 2014).

Each screening method has its own advantages and disadvantages as shown in table 7. The Community Reference Laboratory Residues (CRL) classifies screening methods by different means as follows:

2.4.6.1 CLASSIFICATION BY DETECTION PRINCIPLE

− Biological methods detect cellular responses to analytes (e.g. oestrogenic effect, inhibition of bacterial growth, cellular effect, hormonal effect). These methods are not selective and can cover several chemical classes of active analytes (e.g. hormones, antimicrobials). They do not allow the identification of individual analyte.

− Biochemical methods detect molecular interactions (e.g. antigens, proteins) between analytes and antibodies or receptor proteins (ELISA, RIA). Chemical labelling of either the analyte or antibody/receptor allows the interaction to be monitored and measured. These methods are either selective for a family of analytes having related molecular structures or are sometimes analyte specific.

− Physicochemical methods distinguish the chemical structure and molecular characteristics of analysts by separation of molecules (e.g. TLC, GC, HPLC) and the detection of signals related to molecular characteristics (e.g. UV- DAD, MS, tandem MS). They are able to distinguish between similar molecular structures and allow the simultaneous analysis of several analysts (CLRs, 2010).

Table (7): Advantages and disadvantages of some screening methods

Test	Advantages	Disadvantages
Elisa	• Easy to use • Availability for a good number of specific compounds. • Availability for families of compounds (e.g. sulfanomides, estilbenes). • Large number of samples (42) per kit for a single analyte. • Reduced time to obtain the results (2-2.5 h for most kits). • High sensitivity and specificity. • Possibility to use within the food processing facility	• Increased cost • Limited storage (few months) under refrigeration. • The need for waste disposal. • Interferences giving some false positives. • Only one kit per residue searched.
Biochip array biosensors	• Easy to use. • Results available in short time. • Multiples residues analyzed in one shot (as many as in an array). • Full automation: higher productivity. • High through-put technique: up to 120 samples per hour and array.	• High operative costs, chips and equipment cost. • Analysis restricted to available chips
HPLC	• Reduced time (few hours) to obtain results. • Sensitive • Automation leading to higher productivity. • Specificity depending on a detector	• Expertise required. • Needs sample preparation (Extraction, filtration, addition of internal standards, etc.). • Expensive
Microbial methods	• Can be used for large surveillance programmers. • Basic laboratory equipment. • Broad spectrum. • Easy to use. • Economical.	• Difficult to standardize preparation procedures. • Some test could not insure MRLs compliance. • Sample preparation required to remove false positives due to protein bacterial inhibitors. • Low sensitivity

2.4.6.2 CLASSIFICATION BY THEIR DEGREE OF QUANTIFICATION

1. Qualitative methods give a yes or no response, with no indication of the concentration of the putative analyte. Examples include:

- bacterial growth inhibition tests which give a result of either no zone or zone of inhibition.

- Inhibition tests which give a color change;

- Immunochemical ligand binding tests, where a response is considered as above or below a Cut-Off Level.

- Chromatographic tests (HPLC, LC-MS/MS), where a peak is considered as present or absent.

2. Semi-quantitative methods give an approximate indication of the concentration of the putative analyte. Examples include:

- Microbial growth inhibition tests where an attempt is made to relate the size of the inhibitory zone to the putative analyte concentration;

- Biochemical tests which include a calibration curve (e.g. ELISA, but only if the test is specific for a single analyte).

- Chromatographic tests, calibrated over a short-range which may not include the sample response.

- Physicochemical test (e.g. HPLC, LC-MS/MS) where the measured method precision characteristics do not meet the requirements for quantitative tests.

3. Quantitative methods meet the same requirements for accuracy, dynamic range, and precision as confirmatory tests. And thus, when

the quantification is required, these methods shall be validated as confirmatory methods, as detailed in the Commission Decision 2002/657/EC (CRLs, 2010).

CHAPTER THREE

Materials and Methods

Chapter three

3 MATERIALS AND METHODS

3.1. SAMPLES

A total of 118 milk samples, of which 79 pasteurized, 14 raw and 25 Ultra Heat Treatment (UHT) were purchased from the market from June 2017 to January 2018 using the production date as the collection criteria.

3.2. SAMPLING

The milk samples were collected by random sampling method for a period of 8 months, using the production date as the collection criteria. The volume of the samples was 100 ml, placed in sterile cups, each labeled with a specific code, sampling date, and production date. Samples were transferred in the ice bag and stored in a freezer at -20 °C until analysis. The heat-treated milk samples were of 6 local different brands and three foreign brands available in the Palestinian market.

3.3. DETECTION OF ANTIBIOTIC RESIDUES

The Delvotest SP NT kit supplied by DSM Food Specialties B.V., (The Netherlands) containing 96 wells microtiter plate was used to detect the antibiotic residue in milk samples. Detection is based on the microbial activity, *Bacillus Stearothemophillus* var. *calidolactis*, in the presence of pH indicator. If there is no AR in milk, the bacteria grow and increases the acidity of the medium, changing its color to yellow, and no color change means the milk sample contaminated with AR residues.

The test was conducted as directed by the manufacturer. Milk samples were melted in a water bath at 23 °C and gently shaken. The aluminum foil was removed from the plate, each test well was given a number representing the sample, then filled with a volume of 0.1ml of milk sample using a disposable one-way pipette, one pipette for each sample. The wells were carefully sealed with an adhesive foil and incubated in a preheated water bath at 64 ± 2 °C, for three hours until the negative sample color changed to yellow, and the results recorded.

3.4. OSMOLALITY TEST

The osmolality of the milk samples was determined by using Vapor Pressure Osmometer (VAPRO 5520).

A 10 microliter specimen is aspirated into a micropipette tip. The specimen is then inoculated into a solute-free paper disc in the sample holder, whereupon the sample holder is pushed into the instrument and the sample chamber is locked. The result appears in 75 seconds on the device screen and then recorded.

3.5. MILK COMPOSITION TEST

The chemical analysis of milk samples was determined by using milk analyzer Lactoscan (Ultrasonic Milk Analyzer) according to the manufacturer`s instructions to determine fat, protein, lactose, SNF, solids, freezing point, and pH of the milk samples.

A volume of 25 ml of milk was poured in the sample holder of the analyzer after being shaken gently. The sample holder was put in the recess of the analyzer. The analyzer sucked in the milk, made the measurements and returned the milk in the milk holder and the display shows the results, then the results were recorded.

3.6. pH TEST

The pH of the milk samples was determined by a pH meter device (JENWAY 3310).

3.7. DATA ANALYSIS

The data obtained from laboratory analysis of milk samples for physicochemical properties and osmolality were analyzed by descriptive and analysis of variance (ANOVA) using IBM SPSS Statistics for Windows, version 20. The data obtained from the antibiotic screening test of milk samples were analyzed using Microsoft Excel (2013).

CHAPTER FOUR

Results and Discussion

Chapter four

4   RESULTS AND DISCUSSION

4.1. ANTIBIOTIC RESIDUE

Delvotest SPNT test was used in this study to screen milk samples for antibiotic residues. It is a qualitative test that provides an indication of the presence of the antibiotics by inhibiting microbial growth. However, the high sensitivity ensures that even trace amounts of contaminating antibiotic residues are detected (Amoxicillin 2.5ppb Cephapirin 5.8 ppb Ampicillin 3.0ppb Penicillin G 1.5 ppb) (Tech news, 2016). It was reported that the two tests of Delvo and Copan can detect milk contamination to Penicillin, Cloxacillin Sodium, Sulfamethazine, Cephalexin and Gentamicin based on the standards set by the European Union. These two tests are simple and low-error (Foruozan *et a*l., 2014; Moghadam *et a*l., 2016; Mahmoudi et al., 2013).

Table (8) shows that 5 (36%) raw milk samples of 14, and 21 of 79 pasteurized milk samples representing (27%) were contaminated with antibiotic residues. None of 25 UHT milk samples were contaminated.

Table (8): Presence of antibiotic residues in the function of heat treatment

Treatment method	NO. Sample	Positive (%)
Raw	14	5 (36 %)
Pasteurized	79	21(27 %)
UHT	25	0(0 %)
TOTAL	118	27(23 %)

The high occurrence of antibiotic residues in milk samples is related to the uncontrolled medicines market, weak monitoring system (smuggling and fraud, medicines abuse), weakness of regulatory for the standards and grades of livestock products, the lack of quality assurance programs (MoA, 2014), insufficient withdrawal period and the costs of analyses (Al-Zuhair,2012). In a study conducted by AL Zuhair in West Bank for the detection of B-lactams in 18 milk samples, and Tetracycline's residues in 16 milk samples of which 4 (22.2%) were above the MRLs for B-Lactams and 3 (18.7%) were above MRLs for Tetracyclines (Al-Zuhair,2012). Alipour *et al*., showed that 19.4% of 187 (154 pasteurized and 33 UHT) milk samples contain residues above the EU-MRLs in Iran (Alipour *et al*., 2013). A study conducted in Azerbaijan by Forouzan *et al*., 848 samples of pasteurized milk analyzed for antibiotic residues, results showed that 30.14% were contaminated and 3.19% of these samples suspected (Forouzan *et al*., 2014). Moghadam *et al.,* reported that 62 (24.8%) of 251 pasteurized milk samples contaminated by antibiotic residues in a study conducted in Iran (Moghadam *et al.,* 2016). In Algeria, a study carried out by Layada *et al*., reported that out of 154 raw milk samples analyzed 39 were positive forming 25.3% of the total samples (Layada *et al*., 2016).

4.1.1 RAW MILK SAMPLES.

Table (9) shows, the highest percentage of contaminated raw milk samples of antibiotic residues was in October (100% 2 of 2), meanwhile august and September, had no positive samples.

Table (9): Percentage of contaminated raw milk samples in the function of sampling time.

Raw Samples		
Month	**No. of Samples**	**Positives (%)**
July	3	1 (33%)
Aug	3	0 (0%)
Sep	2	0 (0%)
Oct	2	2 (100%)
Nov	4	2 (50%)
Total	**14**	**5 (36%)**

Table (10) shows, autumn had the highest number of contaminated samples 4 (67%) of 6, and the lowest in summer 1(13%) of 8 raw milk samples. It was determined that the probability of detecting antibiotic in milk during spring and autumn is higher than that in other seasons (Kaya *et al*., 2010). Moreover, it was noted that in autumn and winter, mastitis occurs more frequently due to climatic changes, and as a result, antibiotic therapy is carried out more often (Grandinaru *et al*., 2011; Rasoli *et al*., 2014).

Table (10): Percentage of contaminated raw milk samples in the function of seasons.

Season	NO. of Samples	Positive, %
Summer	8	1 (13%)
Autumn	6	4 (67%)

4.1.2. HEAT TREATED MILK SAMPLES

Table (11) shows, the highest percentage of contaminated samples with antibiotic residues was on November, 4 (50%) of 8, meanwhile, the lowest number was on June 1(9%) of 11.

Table (11): Percentage of contaminated pasteurized milk samples in the function of months.

Pasteurized		
Month	**No.of Samples**	**Positive (%)**
Jun	12	1 (8%)
July	10	2 (20%)
Aug	11	2 (18%)
Sept	10	3 (30%)
Oct	12	4 (33%)
Nov	8	4 (50%)
Dec	9	3 (33%)
Jan	7	2 (29%)
TOTAL	**79**	**21 (27%)**

In the function of season table (12) shows, the highest percentage of positive pasteurized milk samples was in autumn, (37%), followed by winter (31%), and the lowest was in summer (15%). This is in accordance with the results of Alipour who showed that the contamination rate during (April−June) was 18.5%, and 11.6% was observed during (March-May) (Alipour *et al*., 2013). In addition

Moghadam *et al*., 2016 found that the percentage of positive pasteurized milk samples in the different seasons of the year, was (26.4%), (25.4%), (24.1%) and (17.1%) in winter, autumn, spring, and summer, respectively in a study conducted in Iran over five years. In another study,

evaluation of raw and pasteurized milk samples, Mahmoudi *et al.*, showed that (20.83%) of milk samples taken in spring contaminated with antibiotic residues. Meanwhile, in summer, (37.5%) of samples were positive for antibiotic residue (Mahmoudi *et al.*, 2014). In another study, Mahmoudi *et al.,* found that the winter milk samples (32.50%) had the most contamination with antibiotic residues compared to summer milk samples (25.00%) (Mahmoudi *et al.*, 2013)

Table (12): Percentage of contaminated pasteurized milk samples in function of seasons

Pasteurized		
Season	**No. of Samples**	**Positive (%)**
Summer	33	5 (15%)
Autumn	30	11 (37%)
Winter	16	5 (31%)

The high rates of contamination in pasteurized milk could be asserted that antibiotic residues in milk would not be inactivated by normal pasteurization procedures (71°C for 15 sec) (Moats, 1987; Alipour *et al.*, 2013; Zorraquino, 2010). Moreover, in dairy factories, usually good quality raw milk without antibiotics and preservatives are used to produce fermented dairy products such as yogurt and cheese. In preparation of sterile milk (UHT), high-quality raw milk is also used. As a result, low quality and contaminated milk are directed toward pasteurized milk production lines, which is highly consumed (Mahmoudi *et al.*, 2013).

The highest percentage of contaminated milk samples was from manufacturer (C) as shown in table (13), and this could be asserted that antibiotics may be used as a preservative in order to increase shelf life or to inhibit microbial growth in order to cover poor sanitation (Galib, 2014; Schlemper *et al*, 2017). The high frequency of positive samples reveals the need to establish regular residue testing programs in Palestine.

Table (13): Percentage of contaminated pasteurized milk samples according to manufacturers.

Producer	Sample No.	Positive (%)
A	28	2 (7%)
B	15	3 (20%)
C	17	15 (88%)
D	4	0 (0%)
E	4	1 (25%)
F	8	0 (0%)
G	3	1 (33%)
Total	**79**	**22 (28%)**

4.2. OSMOLALITY

The osmolality of milk is a significant value owing to its being highly preserved. Milk containing additional water has a significantly decreased osmolality, corresponding to an increased freezing point. Osmolality has been used for quality control of milk for quite a while. (Buttel *et al.,* 2008).

Milk samples that contain additional

water have a significantly decreased osmolality, corresponding to an increased freezing point

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Table (14) shows the osmolality average of raw milk samples as 309±37.86 mOsmol/kg, the highest value was 430 mOsmol/kg, and the lowest was 281 mOsmol/kg. Moore reported that the osmolality of whole cows' milk is less than 300 mOsm per kg (Moore *et al.,* 2016) which is not agreed with the current study results.

While the osmolality mean of UHT milk samples was 271.76±7.46 mOsmol/kg, the highest value was 288 mOsmol/kg, and the lowest was 248 mOsmol/kg, and the osmolality average of pasteurized milk samples was 273±24.08 mOsmol/kg, the highest value was 331 mOsmol/kg, and the lowest was 227 mOsmol/kg.

Table (14): Milk samples osmolality according to the treated method.

Treated method	N	Minimum	Maximum	Mean	Std. Deviation
Raw	14	281	430	309	37.87
Pasteurized	79	227	331	273.68	34.09
UHT	25	248	288	271.76	7.46

Table (15) shows milk samples osmolality revealed significant differences ($P<0.01$) between raw milk samples and heat-treated milk samples (pasteurized and UHT) and non-significant differences ($P>0.05$) between pasteurized and UHT milk samples. The cleaning water of the pipeline may be polluting milk with cleaning acid or neutralizing agents (residual salts), this cause a depression of the freezing point corresponds to an increase in osmolality. Milk dilution occurs intentionally by adding water or accidentally by water residues in pipelines when milk processing takes place, which causes depression of milk osmolality corresponds to an increase in freezing point (Buttel *et al.,* 2008).

Table (15): Osmolality t-test of milk samples.

Heat-treated Method	Heat-treated Method	P-Value
Raw	Pasteurized	0.00064
Raw	UHT	0.000062
Pasteurized	UHT	0.26

In Germany, the osmolality of 12 heat-treated milk samples was tested by Buttel *et al.,* and the average was found as 271 mOsmol/Kg, the Min value was 263 mOsmol/kg and the Max was 282 mOsmol/kg. After mixing with 10% water by volume, the average decreased to 242

mOsmol/kg, and after mixing with 50% water, the resulting mean osmolality was 129 mOsmol/kg (Buttel *et al.,* 2008).

According to seasons, table (14) showed that the osmolality average of raw milk samples in summer and winter was 310 and 309 mOsmol/kg respectively. While the osmolality average of pasteurized milk samples in summer was 280 mOsmol/kg which is higher than that in autumn and winter, with 274, 275 mOsmol/kg respectively. Also, the osmolality average of UHT milk samples in summer and spring was the same (273 mOsmol/kg), in winter it was 272 mOsmol/kg and in autumn it was 266 mOsmol/kg, which is the smallest value.

The osmolality of raw milk samples average showed non-significant differences (P>0.05) between seasons and with a confidence interval (289-329) mOsmol/kg. Similarly, the osmolality average of Pasteurized and UHT milk samples showed non-significant differences between seasons and with confidence intervals (274.1-280.2), (268.8-274.9) mOsmol/kg, respectively. Table (16) shows the summary for different types of milk samples osmolality according to seasons.

Table (16): Milk samples osmolality average according to seasons.

Item	Measurements	Summer	Autumn	Winter	Spring	Total	P Value	CI
	Mean±Std	309.88	308			309±37.87	0.95	289-329
Raw	**Min**	281	290					
	Max	430	326					
Pasteurized	**Mean±Std**	280.8±15.7	274.54±13.47	275.6±10		277±13	0.19	274.1-280.2
	Min	262	227	252				
	Max	331	303	299				
UHT	**Mean±Std**	273.17±2.17	266.2±11.41	272±6.47	273.75±7.25	271.76±7.46	0.32	268.8-274.9
	Min	270	248	263	265	248		
	Max	276	275	280	288	288		

4.3 FREEZING POINT

The reported results showed that the freezing point of milk is a useful index for detecting added water (Shipe, 1959; Hanus et al., 2011). Depression of the milk freezing point was often related to an increase in protein and solids content as well as to a decrease in the lactose (Bouisf et al., 2018). Also, freezing point affected by the concentration of lactose and pH (Zagorska et al., 2013; Shipe, 1959).

In the present study, Table (17) shows freezing point average of pasteurized milk samples was (-0.5048±0.0186°C), higher during summer (-0.5048±0.097°C), and lower during winter (-0.5120±0.018°C). These results are lower than those reported by Abd Elrahman et $al.,$ (-0.447°C) for pasteurized milk samples (Abd Elrahman et al., 2009). Similarly, the freezing point average of UHT milk samples during summer was the lowest value (-0.5087±0.01°C), and the highest was in autumn (-0.4974±0.02°C). The freezing point of pasteurized and UHT milk samples revealed non-

significant differences between seasons (P>0.05) and the confidence interval was (-0.4945-0.5223°C), (-0.4994-0.5102°C), respectively. The maximum limits of the freezing point of both raw and heat-treated drinking milk were ≤ –0.520°C according to EU regulations (Buttel, 2009), which is not agreed with the current results.

The highest values of the freezing point are found in summer, and in the early autumn, the reason probably lies in higher milk yields and the decrease in milk component and fat-free dry matter contents. Variability may be induced by exposure of dairy cows to heat stress, stage of lactation and, most importantly, dairy cows' nutrition (Zagorska et al., 2013; Shipe, 1959; Navrátilová et al., 2006).

A total of 295 samples of heat-treated drinking milk was examined by Navratelova over a period of one year, 145 of them were pasteurized milk and 150 were UHT milk. The average freezing point was found as -0.514°C, -0.516°C respectively. (Navratelova et al., 2006). Bouisf et al., showed that the mean of milk FP was -0,521. (Bouisf et al., 2018). Mirzadah et al., reported that the FP average of raw milk samples was -0.56°C (Mirzadah et al., 2010). Moreover, Hanus et al quantified 72,607 bulk raw cow milk samples. The highest FP was in spring (−0.52097 ± 0.004877 °C), the lowest in autumn (−0.52516 ± 0.005725 °C) (Hanus et al., 2011).

4.4 pH

The acidity of milk is due to the amount of casein, phosphate, citrate and carbon dioxide. But then at the end of the bacterial activity the lactic

acid was formed and the milk acidity increased (Ozrenk *et al.,* 2008). In normal cow milk, the pH ranges from 6.6 to 6.8 (Kabui, 2012).

In the present study, the pH average of pasteurized milk samples was found as 6.78±0.29, the max was 7.69 and the min was 6.24 during summer. In autumn the average was 6.65±0.47, the max was 7.22 and the min was 5.37, while in winter the average was 6.7±0.24, the max was 7.11 and the min was 6.31. pH revealed significant differences between seasons (P<0.05) and the confidence interval was (6.59-6.75), see table (17). The average pH of UHT milk samples was the lowest during summer 6.53±0.13, and the highest in autumn (6.74±0.40). These results revealed non-significant differences between seasons (P>0.05) and the confidence interval was (6.54-6.77), see table (18). Milk is usually slightly acidic, with a pH value between 6.5 and 6.7, but if the pH value of cow milk is measured to be above 6.8, it may point out the mastitis disease or a neutralized substance added in milk. If pH value is smaller than 6.5, it means colostrum may be present or bacterial growth or spoilage may be occurred in milk (Pelvan, 2011).

Kabui found that the average milk pH of 6.63 in a study conducted in Kenya. In another study conducted by Abd Elrahman *et al.,* the mean milk PH was 7.02 (Abd Elrahman *et al.,* 2009). Chen *et al.,* found that milk pH was significantly higher in spring than in summer and autumn. (6.73) (Chen *et al.,* 2014). Ozrenk *et al.,* reported that the average milk pH in winter and summer was 6.50±0.205, 6.50±0.472, respectively (Ozrenk *et al.,* 2008).

4.5 MILK COMPONENTS

Cow's milk is an essential part of most daily diets and is a complex mixture of specific bioactive molecules such as proteins, lipids, saccharides, and biologically active substances including immunoglobulins, enzymes, oligosaccharides, hormones, and cytokines. Cow milk composition differs not only between species but also within species, through genetics, farming practices or environment (temperature, humidity, wind) (El-Hamdani *et al.,* 2017). Cow milk production and composition can be directly influenced by the season as it affects feed availability. The different season of the year is often related to different food regimes for cows (Nateghi *et al.,* 2014; Kabil *et al.,* 2015; El-Hamdani *et al.,* 2017).

Heat stress affected both quantity and synthesis of milk components (protein, fat, lactose) (Thomas *et al*., 2015; Bernabucci *et al.,* 2014). Heat stressed animals may have lower levels of blood protein and energy due to the in-efficiencies of rumination and metabolism during this heat challenge. Both blood protein and energy levels can influence milk and milk fat yields. In addition to eating less and drinking more, feeding patterns change during heat stress (Bernabucci *et al.,* 2014).

4.5.1 FAT CONTENT

Table (18) shows, the average of the fat content of pasteurized milk samples was found in summer to be 3.13±0.35%, max was 3.63 and the min was 2.52. In autumn the average was 3.25±0.52%, max was 3.9 and

the min was 1.97. During winter the mean was 3.48±0.54, the max was 4.44 and the min was 2.52. Fat variability depends on several factors such as weather conditions, stage of lactation, and feeding (El- Hamdani *et al.,* 2017).

In the present study, the fat content average in pasteurized milk samples was higher in winter (3.48±0.54), followed by autumn (3.25±0.52%), the lowest was in summer (3.13±0.35%). These results revealed non-significant differences between seasons (P>0.05), and the confidence interval was (3.14-3.36). Similarly, Kabil *et al* found fat content in winter (3.6 ±0.055) higher than in summer (3.1 ± 0.058) (Kabil *et al.,* 2015). Nateghi *et al.,* found that the amount of fat in summer (3.39%) was higher than that in winter(3.41) which is not agreed with the current results (Nateghi *et al.,* 2014). Additionally, minimum fat (4.3) content was observed in summer, and a maximum (5.4) in winter, in a study conducted by Yasmeen *et al.,* (Yasmeen *et al.,* 2012).

Hill reported that fat minimum in August, maximum in October in a study carried out over ten years (1991-2001) in the USA (Hill, 2011). Chin *et al* reported that the autumn period had significantly higher fat content than in other periods which is broadly in line with the UK national statistics (Chin *et al.,* 2014).

The average content of fat in UHT milk samples was higher in spring (3.28±0.23) and the lowest was in summer (2.58±0.66). The current results revealed non-significant differences between seasons (P>0.05), and the

confidence interval was (2.83-3.28). Also, Povel showed that the mean fat content was higher in spring morning 3.91±0.23, and the lower in summer morning 3.51±0.31, (Povel *et al.,* 2011). In Sudan, a study conducted by Galib to investigate raw cow milk composition showed that the average of fat content was found as 5.03±0.04% during the winter season, and 4.30 ±0.6% during the summer season (Ghalib, 2014).

4.5.2 SOLID NOT FAT (SNF) CONTENT

Table (17) shows the highest average content of solid not fat of pasteurized milk samples was in autumn (8.04±0.47%), and the lowest was in winter (7.91±0.44%). The highest average content of solid not fat of UHT milk samples was in autumn (8.01±0.47%) and the lowest was in winter (7.85±0.51). Solid not fat average of pasteurized and UHT milk samples showed non-significant differences between seasons (P>0.05), and the confidence interval was (7.9-8.1), (7.78-8.14), respectively. Kunda *et al.,* reported that the average SNF content of pasteurized milk was 8.6% (Kunda *et al.*, 2015). Our results were lower than those revealed by Thomas *et al* which showed that the average SNF in winter was 8.5% and in summer 8.4% (Thomas *et al.,* 2015). Moreover, the results obtained by Abd Elrahman *et al.,* showed the average SNF content of raw milk was 8.58±0.035, and pasteurized 7.93±0.007 which is almost agreed with the current study (Abd Elrahman *et al.,* 2009).

4.5.3 PROTEIN

Table (17) shows the highest average protein content of pasteurized milk samples was in autumn (2.96±0.17), followed by summer 2.94±0.2, and the lowest was in winter 2.92±0.17. Meanwhile, the protein content average of UHT milk was higher in spring (3.18±0.53), and the lowest during the winter (2.87±0.19) table(18). Protein content average of pasteurized and UHT milk samples revealed no significant differences between seasons (P>0.05), and the confidence interval was (2.91-2.99), (2.87-3.11), respectively. Similarly, El- Hamdani *et al.,* found the average protein was 3.6 and there were no differences between seasons (El-Hamdani *et al.,* 2017). Kabil *et al.,* found that there is a variation between seasons and the highest average value was in winter 3.5 ± 0.046, and the lowest was in spring (3.0. ±0.045) (Kabil *et al.,* 2015) which is not agreed with the current study. In another study Nateghi *et al.,* showed that the amount of protein contained in summer milk was higher than in winter milk as its amounts in summer and winter milk were 3.71% and 3.01%, respectively (Nateghi *et al.,* 2014). Additionally, the highest protein content (3.22) was in winter, and the lowest (2.3) in the summer season (Yasmeen *et al.,* 2012). The milk protein content is affected by seasonal changes and locality and its variability is known to be higher than that of fat content (El-Hamdani *et al.,* 2017).

Similarly, Povel *et al.,* showed that the highest protein content was in spring morning 3.41±0.05, and the lowest was in summer morning

3.16±0.11 (Povel *et al.,* 2011), and also Chin *et al.,* reported the higher protein content was observed in spring compared to the summer and autumn periods (Chin *et al.,* 2014

4.5.4 LACTOSE

Table (17) shows the maximum average content of lactose in pasteurized milk samples was during autumn (4.41± 0.26), and the minimum was during summer (4.33±0.49). Similarly, the highest average content of lactose in UHT milk samples was during autumn, and the lowest was during winter and spring, see table (18). The average content of pasteurized and UHT milk samples revealed non-significant differences between seasons (P>0.05), and the confidence interval was (4.36-4.42), (4.27-4.48) respectively. Yasmeen *et al.,* found that the minimum average lactose was in summer (4.93), and the maximum in winter (6.26) (Yasmeen *et al.,* 2012). The lactose content of milk in summer and winter was reported by Nategi *et al.,* as 4.61% and 4.58% respectively (Nateghi *et al.,* 2014). Ghalib showed that the average lactose content in milk samples was 4.62 ±0.2 in winter and 4.72±0.2 in summer (Galib, 2014).

4.5.5 SOLIDS (ASH)

The average content of solids in pasteurized milk samples during summer, autumn and winter did not change and was found as 0.65±0.04.

The average content of solids in UHT milk samples was the highest during summer and autumn (0.65±0.04), and the lowest during winter and

spring (0.64±0.04). These results revealed non-significant differences between seasons (P>0.05). The confidence interval for pasteurized and UHT milk samples was (0.64-0.66), (0.63-0.66), respectively.

Abd Elrahman *et al.,* found that the average solid content in raw and pasteurized milk samples was 0.778±0.003, 0.718±0.001, respectively (Abd Elrahman *et al.,* 2009).

Table (17): Physiochemical properties of pasteurized milk samples according to seasons.

Item	NO. of Samples	31	30	18			
	Measurements	Summer	Autumn	Winter	Total	P-value	CI
Fat %	Mean±Std	3.12±0.35	3.25±0.52	3.48±0.54	3.25±0.48	0.32	3.14-3.36
	Min	2.52	1.97	2.52			
	Max	3.64	3.9	4.45			
SNF %	Mean±Std	8.03±0.49	8.04±0.47	7.92±0.44	8±0.47	0.65	7.9-8.1
	Min	7.15	7.07	7.07			
	Max	9.91	9.19	8.89			
Ash %	Mean±Std	0.65±0.04	0.65±0.04	0.65±0.04	0.65±0.04	0.82	0.64-0.66
	Min	0.58	0.58	0.57			
	Max	0.81	0.75	0.72			
Lactose %	Mean±Std	4.33±0.27	4.41±0.26	4.35±0.24	4.36±0.26	0.053	4.31-4.42
	Min	3.9	3.89	3.9			
	Max	5.45	5.05	4.89			
Protien %	Mean±Std	2.94±0.2	2.96±0.17	2.92±0.17	2.95±0.19	0.4	2.91-2.99
	Min	2.62	2.59	2.59			
	Max	3.63	3.36	3.25			
FP (-)	Mean±Std	0.5048±0.0976	0.51±0.0249	0.512±0.0186	0.5084±0.0631	0.12	0.4945-0.5223
	Min		0.42	0.47			
	Max	0.62	0.56	0.56			
pH	Mean±Std	6.78±0.29	6.56±0.47	6.70±0.24	6.67±0.37	0.046	6.59-6.75
	Min	6.24	5.37	6.31			
	Max	7.67	7.22	7.11			

Table (18): Physiochemical properties of UHT milk samples according to seasons.

Item	No. of Samples	7	7	6	6			
	Measurements	Summer	Autumn	Winter	Spring	Total	P-Value	CI
Fat %	**Mean±Std**	2.58±0.67	3.2±0.33	3.03±0.7	3.28±0.23	3.06±0.53	0.19	2.84-3.28
	Min	1.78	2.67	1.78	2.83			
	Max	3.37	3.59	3.66	3.47			
SNF %	**Mean±Std**	7.98±0.57	8.01±0.46	7.85±0.51	7.99±0.26	7.96±0.45	0.92	7.78-8.14
	Min	7.07	7.48	6.03	7.72			
	Max	8.89	8.82	8.36	8.4			
Ash %	**Mean±Std**	0.65±0.04	0.65±0.04	0.64±0.04	0.64±0.02	0.65±0.04	0.97	0.63-0.66
	Min	0.58	0.6	0.56	0.62			
	Max	0.72	0.72	0.68	0.67			
Lactose %	**Mean±Std**	4.38±0.31	4.41±0.24	4.35±0.34	4.35±0.2	4.38±0.26	0.96	4.27-4.48
	Min	3.89	4.1	3.81	4.23			
	Max	4.89	4.85	4.84	4.51			
Protien %	**Mean±Std**	2.93±0.20	2.97±0.16	2.87±0.19	3.18±0.53	2.99±0.31	0.32	2.87-3.11
	Min	2.59	2.73	2.53	2.86			
	Max	3.25	3.22	3.05	4.23			
FP (-)	**Mean±Std**	0.5087±0.0059	0.4974±0.02	0.5057±0.0059	0.5080±0.0159	0.5048±0.014	0.61	0.4994-0.5102
	Min	0.52	0.52	0.51	0.54			
	Max	0.5	0.46	0.5	0.49			
pH	**Mean±Std**	6.53±0.13	6.74±0.4	6.68±0.26	6.65±0.35	6.65±0.30	0.61	6.54-6.77
	Min	6.36	6.08	6.41	6.32			
	Max	6.74	7.1	7017	7.15			

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1. CONCLUSION

From the present study, the following conclusion could be drawn:

- Physicochemical properties such as fat, solids not fat, acidity, lactose, and protein content showed that the milk samples were of good quality .

- Physiochemical properties of milk (fat, protein, lactose, SNF, and total solids, PH, FP) are affected by seasonal variation.

- As a result of the antibiotic screening test, the study revealed that 5(36%) of 14 raw milk samples contained antibiotic residues, and 21(27) of 79 pasteurized milk samples were contaminated by antibiotic residues. None of the 25 UHT milk samples was contaminated by antibiotic residues.

- The osmolality of milk affected by seasonal variation

5.2. RECOMMENDATIONS

The following measures must be put into consideration in order to prevent or reduce the incidence of antibiotic residues in milk:

1- The farmers should not have access to veterinary antibiotics, whose distribution should be regulated.

2- The uses of antibiotics for the treatment of mastitis or any other infection should be under veterinary inspection.

3- An established network of scientists and regulators to implement a residue control program.

4- Drug screening tests on-farm should be used.

5- More studies on drug residues in food animal and establishment of suitable regulations and inspection systems are needed to reduce the risks of antibiotic and other drug residues for public health

6- Adequate withdrawal period should be observed in all milking cows following the therapeutic use of antibiotics.

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جامعة النجاح الوطنية

كلية الدراسات العليا

تقييم جودة الحليب والكشف عن متبقيات المضادات الحيوية في عينات حليب من السوق الفلسطيني

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الملخص

أجريت هذه الدراسة لتقييم جودة الحليب والكشف عن متبقيات المضادات الحيوية في السوق الفلسطيني خلال 8 أشهر، ابتداء من شهر حزيران 2017 الى كانون الثاني 2018. تم جمع 118 عينة حليب من السوق الفلسطيني، منها 14 خام و 79 مبستر و 25 مبستر بالحرارة الفائقة UHT، وذلك باستخدام تاريخ الانتاج كمعيار لجمع العينات. جرى فحص العينات للتحري عن تواجد بقايا المضادات الحيوية باستخدام اختبار Delvotest SP-NT ، وتم قياس الأسمولية الخاصة بها باستخدام جهاز قياس الضغط البخاري VAPRO. بالإضافة الى ذلك، تم تقييم عينات الحليب المبستر والحليب عالي البسترة بناء على الخواص الفيزيائية والكيميائية (الدهون، الجوامد الصلبة اللادهنية، البروتين،اللاكتوز، المواد الصلبة، درجة التجمد ودرجة الحموضة)، وذلك باستخدام جهاز Lactoscan ومقياس درجة الحموضة pH .

أظهرت نتائج اختبار فحص متبقيات المضادات الحيوية SP-NT ان 36% من عينات الحليب الخام و 27% من العينات المبسترة تحتوي على متبقيات المضادات الحيوية ولم يتم العثور على متبقيات المضادات الحيوية في اي عينة من الحليب عالي البسترة.

أظهرت النتائج ان معدل محتوى الدهون والمواد الصلبة (الرماد)، 3.38%، 0.65 % على التوالي، في عينات الحليب المبستر أعلى خلال فصل الشتاء مقارنة بباقي الفصول. علاوة على ذلك كان معدل درجة التجمد ودرجة الحموضة pH، -0.5048، 6.78 درجة مئوية على التوالي، أعلى خلال فصل الصيف. علاوة على ذلك كان معدل SNF، البروتين واللاكتوز أعلى في الخريف (8.04%، 2.96% ،4.41% على التوالي).

أظهرت النتائج عدم وجود فروق معنوية (P>0.05) لنسبة الخواص الفيزيائية والكيميائية (الدهون، الجوامد الصلبة اللادهنية، البروتين،اللاكتوز، المواد الصلبة، درجة التجمد) لعينات الحليب المبستر بين الفصول، بينما كان هناك فروق معنوية لنسبة pH (P<0.05).

كما اظهرت النتائج ان معدل نسبة الدهون والبروتين في عينات الحليب عالي البسترة UHT كان أعلى في فصل الربيع (3.28%، 3.18%، على التوالي)، أما معدل نسبة المواد الصلبة ودرجة التجمد فقد كانت أعلى في الصيف (0.65%، -0.5087% درجة مئوية، على التوالي). علاوة على ذلك ،كان معدل نسبة المواد الصلبة اللادهنية SNF وpH أعلى في الخريف (8.01% ،6.74%، على التوالي). أظهرت النتائج عدم وجود فروق معنوية (P>0.05) لنسبة الخواص الفيزيائية والكيميائية (الدهون، الجوامد الصلبة اللادهنية، البروتين،اللاكتوز، المواد الصلبة، درجة التجمد،pH) لعينات الحليب عالي البسترة UHT بين الفصول.

كان متوسط الاسمولية لعينات الحليب الخام 309 ±37.86 موسمول/كغم، ومتوسط الاسمولية لعينات الحليب المبستر كانت 273 ±24.08 موسمول/ كغم، بينما بلغت الاسمولية لعينات الحليب عالي البسترة UHT 271.76 ±7.46 موسمول/كغم. كما اظهرت النتائج وجود فروق معنوية (P<0.05) لنسبة الاسمولية بين عينات الحليب الطازج والحليب المعالج حراريا (المبستر وعالي البسترة).

وقد خلصت الدراسة الحالية إلى أن الخواص الفيزيائية والكيميائية للحليب وكذلك الأسمولية تأثرت باختلاف الفصول، كما أظهرت الدراسة أن النسبة المئوية لعينات الحليب الخام الملوثة بمتبقيات المضادات الحيوية أعلى مقارنة مع عينات الحليب المعالج حراريا.