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HEALTH - PROMOTING BENEFITS (ANTIOXIDANTS, ANTIHYPERTENSIVE, ANTIDIABETIC) AND PROBIOTIC SURVIVABILITY OF FERMENTED CAMEL MILK AFTER IN - VITRO DIGESTION BY INFOGEST 2.0: COMPARATIVE STUDY WITH FERMENTED BOVINE MILK

Ahlam Faisal Al Hammadi
United Arab Emirates University

United Arab Emirates University

College of Agriculture and Veterinary Medicine

Department of Food Science

HEALTH - PROMOTING BENEFITS (ANTIOXIDANTS, ANTIHYPERTENSIVE, ANTIDIABETIC) AND PROBIOTIC SURVIVABILITY OF FERMENTED CAMEL MILK AFTER IN - VITRO DIGESTION BY INFOGEST 2.0: COMPARATIVE STUDY WITH FERMENTED BOVINE MILK

Ahlam Faisal Abdalla Ahmed Al Hammadi

This thesis is submitted in partial fulfilment of the requirements for the degree of Master of Science in Food Science

Under the Supervision of Professor Mutamed Ayyash

November 2021

Declaration of Original Work

I, Ahlam Faisal Abdalla Ahmed Al Hammadi, the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of this thesis entitled "*Health - Promoting Benefits (Antioxidants, Antihypertensive, Antidiabetic) and Probiotic Survivability of Fermented Camel Milk After in - vitro Digestion by Infogest 2.0 : Comparative Study with Fermented Bovine Milk*", hereby, solemnly declare that this thesis is my own original research work that has been done and prepared by me under the supervision of Dr. Mutamed Ayyash, in the College of Agriculture and Veterinary Medicine at UAEU. This work has not previously formed the basis for the award of any academic degree, diploma or a similar title at this or any other university. Any materials borrowed from other sources (whether published or unpublished) and relied upon or included in my thesis have been properly cited and acknowledged in accordance with appropriate academic conventions. I further declare that there is no potential conflict of interest with respect to the research, data collection, authorship, presentation and/or publication of this thesis.

Student's Signature: *Ahlam Hammadi* Date: 09.10.2021

Advisory Committee

1) Advisor: Mutamed Ayyash

Title: Associate Professor

Department of Food Science

College of Agriculture and Veterinary Medicine

2) Co - advisor: Akmal Nazir

Title: Associate Professor

Department of Food Science

College of Agriculture and Veterinary Medicine

3) Member: Afaf Kamal Eldin

Title: Professor

Department of Food Science

College of Agriculture and Veterinary Medicine

Approval of the Master Thesis

This Master Thesis is approved by the following Examining Committee Members:

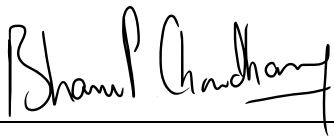
1) Advisor (Committee Chair): Dr. Mutamed Ayyash
   Title: Associate Professor of Food Science
   Department of Food Science
   College of Agriculture and Veterinary Medicine
   Signature _____ Date 25/01/2022

2) Member: Prof. Afaf Kamal Eldin
   Title: Professor of Food Science
   Department of Food Science
   College of Agriculture and Veterinary Medicine
   Signature _____ Date 26/01/2022

3) Member (External Examiner): Dr. Belal J. Muhialdin
   Title: Senior Lecturer
   Department of Faculty of Food Science and Technology
   Institution: Universiti Putra Malaysia
   Signature _____ Date 01/02/2022

This Master Thesis is accepted by:

Dean of the College of Agriculture and Veterinary Medicine: Dr. Bhanu Chowdhary

Signature  Date 06/04/2022

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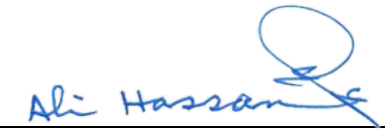
Signature  Date 06/04/2022

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Abstract

This study investigated probiotic survival and biological functionality of the bio accessible fraction of fermented Camel milk (FCM) and fermented Bovine milk (FBM) after *in - vitro* digestion using INFOGEST 2.0 model. Both Camel and Bovine milk were fermented by starter culture only (SC), probiotic *Lactococcus lactis* (Pro), and a mixture of SC+Pro followed by storage for 21 days. The obtained water - soluble extract (WSE), excreta, and bio accessible fraction during the *in - vitro* digestion were assessed for it's health benefits. The probiotic survival, proteolysis rate, antioxidant activities, ability to inhibit α - Amylase and α - Glucosidase, and ACE - inhibitions were assessed. For all starter cultures, the highest reduction in the viable numbers was noted after the gastric step in both fermented Camel and Bovine milk. The bacterial count reduction ranged from ~ 0.9 to 3.3 logs and ~ 2.2 to 3.6 in fermented Camel milk and from ~ 2.3 to 4.3 logs and 3.1 to 4.6 logs in fermented Bovine milk after gastric and intestinal steps, respectively. After 21 days of storage, the OPA absorbances in WSE, centrifuged excreta, and bio accessible fractions were comparable in all fermented milk types with slightly higher in fermented Bovine. A slight difference could be observed in the protein bands (SDS - PAGE) especially both Camel and Bovine milk fermented by *L. lactis* C47 (Pro). At day 1, the bio accessible fraction and the excreta from both fermented Camel and Bovine milk had a higher ABTS scavenging than the WSE for all fermented products (SC, SC+Pro, and Pro), despite that fact that the protein content of the bio accessible fraction was notably lower than for the other two fractions. The inhibitory activities for the WSE increased by the end of the storage period, for all treatments in both types of fermented milk, but this was not observed for the bio accessible fraction. Among all treatments, bio accessible from Camel milk fermented by SC or SC+Pro bacteria had the highest ACE - inhibitory effect (86.7%). Compared to the WSE of fermented Bovine milk before *in - vitro*, that of fermented Camel milk possessed greater biological functionality. The potential probiotic *L. lactis* exhibited reasonable survival rate in fermented Camel milk during the *in - vitro* digestion.

Keywords: Camel Milk, Anti - Cancer, Anti - Hypertensive, Antioxidant.

Title and Abstract (in Arabic)

الفوائد المعززة للصحة (مضادات الأكسدة ، ومضادات لإرتفاع ضغط الدم ، ومضادات مرض السكري) وبقاء بروبيوتيك لحليب الإبل المخمر بعد الهضم داخل الفيترو بواسطة إنفوجيست 2.0: دراسة مقارنة مع الحليب البقري المخمر

الملخص

بحثت هذه الدراسة في بقاء الكائنات الحية المجهرية والوظيفة البيولوجية للجزء الذي يمكن الوصول إليه بيولوجيًا من حليب الإبل المخمر (FCM) وحليب الأبقار المخمر (FBM) بعد الهضم في المختبر. تم تخمير كل من حليب الإبل والأبقار بواسطة الزراعة البادئة فقط (SC) ، بروبيوتيك (Pro) (Lactococcus lactis) ، ومزيج من SC+Pro متبوعًا بالتخزين لمدة 21 يومًا. تم استخدام الحليب غير المخمر كعنصر تحكم. في اليوم الأول واليوم 21 من التخزين ، تم هضم عينات الحليب المخمر في المختبر باستخدام نموذج INFOGEST 2.0 . علاوة على ذلك ، تم تقييم المستخلص القابل للذوبان في الماء (WSE) والفضلات والجزء الذي يمكن الوصول إليه بيولوجيًا أثناء الهضم في المختبر لفوائده الصحية. تم تقييم بقاء البروبيوتيك ، ومعدل تحلل البروتين ، والأنشطة المضادة للأكسدة ، والأنشطة المضادة لمرض السكر ، ومثبطات الإنزيم المحول للأنجيوتنسين. بالنسبة لجميع البكتيريا ، لوحظ أعلى انخفاض في أعداد قابلة للحياة بعد خطوة المعدة في كل من حليب الإبل والأبقار المخمر. تراوح انخفاض العد البكتيري من 0.9 إلى 3.3 سجل و 2.2 إلى 3.6 في حليب الإبل المخمر ومن 2.3 إلى 4.3 سجل و 3.1 إلى 4.6 سجل في حليب الأبقار المخمر بعد خطوات المعدة والأمعاء ، على التوالي. بعد 21 يومًا من التخزين ، كانت امتصاصات OPA في WSE ، والفضلات الطاردة المركزية ، والكسور التي يمكن الوصول إليها بيولوجيًا قابلة للمقارنة في جميع أنواع الحليب المخمر مع ارتفاع طفيف في الأبقار المخمرة. يمكن ملاحظة اختلاف طفيف في نطاقات البروتين (SDS - PAGE) وخاصة حليب الإبل والأبقار المخمر بواسطة *L. lactis* C47 (Pro). في اليوم الأول ، كان للجزء الذي يمكن الوصول إليه بيولوجيًا والإفرازات من كلٍّ من حليب الإبل والأبقار المخمر نسبة عالية من ABTS في الكسح مقارنةً بـ WSE لجميع المنتجات المخمرة (SC و SC + Pro و Pro)، على الرغم من حقيقة أن محتوى البروتين في المواد التي يمكن الوصول إليها بيولوجيًا كان الكسر أقل بشكل ملحوظ من كسرين آخرين. ازدادت الأنشطة المثبطة لـ WSE بنهاية فترة التخزين ، لجميع المعالجات في كلا النوعين من الحليب المخمر ، ولكن لم يتم ملاحظة ذلك بالنسبة للجزء القابل للنفاذ البيولوجي. من بين جميع العلاجات ، كان الوصول الحيوي من حليب الإبل المخمر بواسطة بكتيريا SC أو SC + Pro أعلى تأثير مثبط للإنزيم المحول للأنجيوتنسين (86.7%) بالمقارنة مع WSE لحليب الأبقار المخمر من قبل في المختبر ، فإن حليب الإبل المخمر يمتلك وظائف بيولوجية أكبر. المحتمل Lc الكائنات الحية المجهرية. أظهرت اللاكتيس معدل بقاء معقول في حليب الإبل المخمر أثناء عملية الهضم في المختبر.

مفاهيم البحث الرئيسية: حليب الإبل ، مضاد للسرطان ، مضاد لارتفاع ضغط الدم ، مضاد للأكسدة.

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Last but not the least I would like to appreciate the emotional and social support my husband and my friends have given to me for completing this journey.

Dedication

To my beloved parents and family

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List of Abbreviations

$(NH4)_2CO_3$	Ammonium Carbonate
ABTS	2,2`-Azino - Bis(3 - Ethylbenzo - Thiazoline - 6 - Sulphonic Acid)
ACE	Angiotensin Converting Enzyme
$CaCl_2$ $(H_2O)_2$	Calcium Chloride Tetrahydrate
DNS	3,5 - Dinitrosalicylic Acid
DPPH	1,1 - Diphenyl - 2 - Picrylhydrazyl
GCC	Gulf Cooperation Council
HCl	Hydrochloric Acid
HHL	Hippurly - Histidyl - Leucin
HPLC	High - Performance Liquid Chromatography
KCl	Potassium Chloride
KH_2PO_4	Potassium Dihydrogen Phosphate
LAB	Lactic Acid Bacteria
Lb.	*Lactobacillus*
$MgCl_2(H2O)_6$	Magnesium Chloride Hexahydrate
MRS	De Man, Rogosan And Sharpe
Na_2CO_3	Sodium Carbonate
NaCl	Sodium Chloride
$NaHCO_3$	Sodium Bicarbonate
NaOH	Sodium Hydroxide
ND	Not Detected
OPA	O - Phthalaldehyde

PBS	Phosphate Buffered Saline
PCA	Principal Component Analysis
pNPG	Pnitrophenyl A - D - Glucopyranosid
Pro	Probiotic *Lactococcus Lactis*
SC	Starter Culture - *Lactobacilli*
SDS	Sodium Dodecyl-Sulfate Polyacrylamide Gel Electrophoresis
SGF	Simulated Gastric Fluids
SIF	Simulated Intestinal Fluids
spp.	Species
SSF	Simulated Digestion Fluids
subsp.	Subspecies
TA	Titratable Acidity
UV	Ultraviolet
UV - Vis	Ultraviolet Visible
WSE	Water Soluble Extract

Chapter 1: Introduction

1.1 Overview

Camels belong to the Camelidae family (Khan *et al.*, 2003). The word ‘Camel’ is believed to have Greek ('Kermal') or Sanskrit origins ('Kreluk') (Khan, Arshad and Riaz, 2003). The animal is nicknamed as the 'Ship of the desert.' The breed differentiation varies based on body conformation, size, color, function, and habitat. The Camelid family is broadly classified into *Camelus*, *Lama,* and *Vicugna* genera (Ali, Baby and Vijayan, 2019). The main two species of large camelids are the single - humped Camel *Camelus dromedaries* and the two - humped Camel *Camelus bactrianus.* The dromedary camels are found in Asia and in northern part of Africa, and are colloquially termed as 'Arabian Camels’.

1.2 Camel Population

It has been evaluated that around 37 million camels exist worldwide (FAOSTAT, 2018). The Food Agriculture Organization has determined the top ten countries with Camel distribution to be Somalia (7.15 million) followed by Sudan (4.79 million), Kenya (2.93 million), Niger (1.72 million), Chad (1.55 million), Mauritania (1.39 million), Ethopia (1.16 million), Pakistan (1.02 million), Mali (0.97 million) and Yemen (0.46 million) (FAOSTAT, 2018).

1.3 Popular Camel Breeds

The genetic makeup of all Camel breeds is unique. However, the various breeds are differentiated based on colour phenotypes. Some of the most popular breeds are Aouadi, Asail, Awrk, Hadhana, Hamor, Maghateer, Majaheem, Safrah, Saheli, Shaele, Shageh, Sofor, Waddah and Zargeh. Interestingly, scientists have correlated colour

phenotypes with certain economic and behavioural traits (Ali, Baby and Vijayan, 2019).

1.4 Camel Milk

The Camel udder is four - quartered with four teats. It is a usual practice to do the milking by hand. Nevertheless, machine milking is also practiced widely (Park and Haenlein, 2008). The milk yield varies based on factors like forage quantity and quality, watering frequency, climate, breeding age, parity, milking frequency, nursing, milking method, health, reproductive status, and individual merit. The lactation curve of the Camel in the first seven months reaches its peak, after which a fair decline is observed (Park and Haenlein, 2008). The average pH value of camel milk ranges from 6.5 to 6.7 (Farah, 1993), which is very similar to cow milk (Visentin *et al.*, 2017). Generally, the specific gravity, fat, protein lactose and ash are higher in Bactrian than Dromedary milk (Park and Haenlein, 2008).

The Camel colostrum (vital for the new born health) is rich in immune enhancing biomolecules, has low viscosity, is sweet and sharp in taste and has a yellowish - white colour (Bernabucci, Basiricò and Morera, 2013). The colostrum composition varies based on Camel type with the Bactrian colostrum being higher in lactose, protein, and ash than their Dromedary counterparts (Park and Haenlein, 2008). The colostrum generally has high levels of total solids, total proteins, ash, and chlorides, but is low in lactose (Bernabucci *et al.*, 2013).

1.4.1 Protein Composition

Proteins are broadly classified into two categories, caseins and Whey. The average casein and Whey protein content in Camel milk varies between 1.9 - 2.3% and 0.7 -

1.0% (Farah, 1993). Whey proteins can be further classified into β - lactoglobulin, α - lactalbumin, serum albumin, lactoferrin and immunoglobulins (Hailu *et al.*, 2016). Camel milk is deficient in β - lactoglobulin, has trace amounts of lactoferrin and is rich in serum albumin and α - lactalbumin (Hailu *et al.*, 2016). Camel lactoferrin is unique as it exhibits both iron scavenging and iron releasing properties, simultaneously (Hailu *et al.*, 2016). The immunoglobulins namely IgG2 and IgG3 found in Camel Whey are not typically present in Bovine milk (Hailu *et al.*, 2016). The colostrum of Camel and Bovine milks also varies, however, serum albumin and β - lactoglobulin are the most dominant in both the milks, respectively (Hailu *et al.*, 2016).

With regards to the casein fraction, the α_{S1} - casein, α_{S2} - casein and κ - casein is lower in Camel milk compared to Bovine milk (Hailu *et al.*, 2016). However, the β - casein content is significantly higher (Hailu *et al.*, 2016). Camel α - casein is deficient in non - essential amino acids except Arginine compared to its Bovine counterpart (Hailu *et al.*, 2016). Moreover, the diameter of Camel casein micelle is larger than ruminant casein (Hailu *et al.*, 2016).

The protein and Nitrogen fraction in Camel milk is similar to that of cow milk. However, Camel milk is higher in the Non protein nitrogen fraction than its Bovine counterpart. Furthermore, casein nitrogen decreases in the pre - lactation period, increases in mid - lactation, and then decreases by the end of lactation. As lactation progresses, Whey protein nitrogen decreases, and non - protein Nitrogen increases (Park and Haenlein, 2008).

1.4.2 Lipids

Lipids are an excellent energy source and are a medium needed for the absorption of fat - soluble vitamins. The fat content in Camel milk varies between 2.7 to 3.6%. Short - chain fatty acids (C4 - C12) are present in small amounts in Camel milk. Moreover, phospholipids in Camel milk are higher in linoleic acid long - chain polyunsaturated fatty acids (Farah, 1993). Camel milk is lower in saturated fatty acids and cholesterol compared to Bovine milk (Bakry *et al.*, 2021). However, it is higher in Monounsaturated Fatty Acids, Polyunsaturated Fatty acids and Phospholipids (Bakry *et al.*, 2021). The Camel milk fat globule is the smallest in terms of size as compared to other milks (Bakry *et al.*, 2021).

1.4.3 Minerals and Vitamins

Generally, milk is a rich source of chlorides, phosphates and citrates of Sodium, Calcium, and Magnesium. The chloride and citrate content of Camel milk is similar to cows' milk (Farah, 1993).

Camel milk is deficient in B - vitamins namely, B_1, B_2, folic acid, and pantothenic acid but rich in Niacin and Vitamin C compared to cow milk. The Vitamin B_6 and B_{12} content of Camel milk is similar to cows' milk (Park and Haenlein, 2008).

1.5 Camel Milk Products

In rural areas of Africa, Asia, and the Middle East, raw and fermented Camel milk products are an important part of the diet (Brezovečki *et al.*, 2015).

1.5.1 Shubat (Chal)

Shubat is a traditional sparkling white fermented milk with a sour taste. It is popular in Turkey, Kazakhs, tan, and Turkmenistan. The preparation of Shubat involves fermenting Camel milk in skin bags or ceramic jars. Fresh milk is added in batches to the previously soured milk and stored for 3 to 4 days after every subsequent addition (Yam *et al.*, 2015). Lactic Acid Bacteria (LAB) (*Lactobacillus casei*, *Streptococcus thermophilus*) and *Yeasts* are common contaminants from air, water and the holding containers (Yam *et al.*, 2015). The average pH of Chal (ranging from 3.8 to 4.5) desists the growth of other microorganisms and this allows a competitive edge for the LAB and *Yeasts* to grow (Yam *et al.*, 2015). Upto thirty five yeast species belonging to 18 genera were isolated from Chal samples in one study, with the predominant LAB being *Lactobacillus plantarum*, *Lactobacillus hilgardii* and Leuconostoc (Yam *et al.*, 2015). The fat, protein content, total solid, calcium and phosphorus content in Chal was determined to be 5.82 ± 0.27%, 3.07 ± 0.073%, 12.24 ± 0.16%, 103.29 ± 3.87% and 10.25 ± 0.1%, respectively (Salami, Tamaskani Zahedi and Moslehishad, 2016). It was also observed to exhibit high antioxidant activity (Salami, Tamaskani Zahedi and Moslehishad, 2016).

1.5.2 Suusac (Susa)

Suusac is a traditional smoked fermented milk commonly consumed in Eastern Africa, Kenya, and Somalia. Using mesospheric dairy cultures to ferment the Camel milk and make Suusac has been attempted previously (Brezovečki *et al.*, 2015).

1.5.3 Gariss/ Hameedh

Gariss is a type of fermented camel milk product commonly consumed in Sudan and Somalia. To the camel milk, onion bulb and black cumin is added. The fermentation is done in goat skin bags covered with moistened green grass. The bag is placed on the saddle of camels, whose rough walk results in thorough shaking of the milk. The total solid, fat, protein and carbohydrate content of Gariss was determined to be 10 - 11%, 2.8 - 5%, 2.3 - 3.4% and 5%, respectively (Shori, 2012). The average pH and Total acidity, Ethanol content are 4.42 ± 0.21, 1.72 ± 0.04% and 1.40 ± 0.03%, respectively (Sulieman, Ilayan and Faki, 2006).

The microflora in Garris is dominated by *Lactobacillus* (*Lactobacillus paracasei* ssp.*paracasei*, *L. fermentum*, *L. plantarum*) followed by *Lactococcus* (*Lactococcus lactis*), *Enterococcus* and *Leuconostocs*. A *Yeast* count of 6.0 ± 0.53 $\log_{10}$ CFU/mL has also been observed (Sulieman, Ilayan and Faki, 2006).

1.5.4 Ititu

Ititu is produced by fermenting camel milk in custom made smoked plant fibre vessels. The vessels are traditionally known as Gorfa. The gorfa is woven, washed with hot water, air dried, rinsed with fresh milk and smoked with the splinters of *Acacia nilotica*. The lid of the vessel is treated with *Ocimum basilicum*. Whey is consistently removed upon milk coagulation using a wooden pipette and a concentrated product is formed (Seifu *et al.*, 2012). The isolated bacteria from the milk samples are dominated by LAB namely *Lactobacillus* (*Lactobacillus plantarum*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus salivarius*), *Lactococcus* (*Lactococcus lactis* subsp.

lactis, Lactococcus lactis subsp. *Cremoris*) and *Enterococcus* (*Enterococcus faecalis*) (Hawaz, 2014).

1.5.5 Cheese

Processing cheese from Camel milk is difficult due to the poor coagulation properties of Camel milk (Yagil, 1982).Efforts have been made to make the cheese using Camel Chymosin and blends of Camel milk and Bovine milk with limited success (Ayyash *et al.*, 2021a; Baig *et al.*, 2022).

1.5.6 Butter

According to previous studies, the same technology used when processing butter from cow milk cannot be used for Camel milk. The high melting point of Camel milk fat makes it difficult to churn the cream at temperatures used for churning cow milk (Brezovečki *et al.*, 2015). In Saharan regions, butter is produced by fermenting Camel milk in goatskin at room temperature for about 12 hours (Yagil, 1982).

1.6 Probiotics

The word “Probiotic” means “for life”. To classify a microorganism as a probiotic, the recent consensus is that the microorganism should be well defined and should exert health benefits (Marco *et al.*, 2021). Probiotics may be bacteria, molds, or yeast (Soccol *et al.*, 2010). Nevertheless, most of the probiotics are bacteria (Singh *et al.*, 2011). *Lactobacillus plantarum*, *Lactococcus lactic,* and *Enterococcus lactis* are some strains naturally found in Camel milk which could potentially act as probiotics (Sharma *et al.*, 2021).

1.6.1 History of Probiotics

The history of probiotics goes to the beginning of the 20th century. A professor at the Paster Institute in Paris, Élie Metchnikoff had hypothesized that the longevity of Bulgarian Peasants was due to their high consumption of fermented milk products. He believed that the beneficial LAB inherent to the fermented food products competed against pathogenic bacteria in the intestine. This resulted in a decrease in overall toxin production and the incidence of infection and disease (Singh *et al.*, 2011).

Various studies indicate a strong association between gut microbiota, probiotics and human health (Kechagia *et al.*, 2013; Deshpande *et al.*, 2017; Tenorio-Jiménez *et al.*, 2020).

1.6.2 Probiotic Classification

LAB can be derived into two groups according to their metabolic pathways, namely Homofermentative and Heterofermentative. Homofermentative LAB ferment sugars under anaerobic conditions to produce sole lactic acid. Heterofermentative LAB ferment sugars to produce ethanol, Carbon dioxide, and lactic acid (Abushelaibi *et al.*, 2017).

1.6.3 Characteristics of Probiotics

To be classified as a probiotic, the microbial strain should be viable, nonpathogenic, nontoxic, capable of surviving the challenging gut environment and be stable under different storage conditions (Singh *et al.*, 2011).

1.6.4 Beneficial Health Effects of Probiotics

Probiotics have been associated with numerous beneficial health outcomes as discussed in detail below (Kechagia *et al.*, 2013).

1.6.4.1 Boosting the Immune System

The relationship between the microbiota and host could either be commensal or symbiotic. Probiotic microorganisms compete with pathogenic bacteria for epithelial binding sites. This prevents the colonization of pathogenic bacteria like *Salmonella* spp. and *E. coli* within the gut (Soccol *et al.*, 2010). Furthermore, probiotics enhance the immune system by activating macrophages, increasing cytokines level, immunoglobulins or natural killer cell activity (Singh *et al.*, 2011). This reduces pathogen growth and indirectly boosts health (Maldonado *et al.*, 2019).

1.6.4.2 Maintaining Positive Mental Health

Literature suggests that probiotics may also help improve mental health (Kerry *et al.*, 2018). Several neuropsychiatric conditions have been associated with dysregulation of the gut microbiota (Sherwin *et al.*, 2016). Probiotics play a positive role by maintaining an appropriate balance of the microbiota and thereby prevent the transmission of any negative signals to be transferred via the vagal or the endocrine pathways to the brain (Ansari *et al.*, 2020).

1.6.4.3 Maintaining Gut Health

Probiotics help to increase the digestibility/ absorption of some nutrients such as carbohydrates, protein and fat (Oliphant and Allen-Vercoe, 2019). They also produce lactic acid, propionic acid, and butyric acid as byproducts of digestion. This maintains an appropriate colonic mucosal pH which aids it in making an unpreferable location

for pathogen colonization (Singh *et al.*, 2011). Probiotics are generally prescribed as complementary therapy in the treatment of antibiotic associated diarrheas and travelers' diarrhea. They have also been associated with beneficial impact on inflammatory bowel diseases, irritable bowel syndrome, colitis, and alcoholic liver disease (Singh *et al.*, 2011).

1.6.4.4 Anticarcinogenic Effect

A previous study has demonstrated that some species of *Lactobacillus* and *Bifidobacterium* spp. may be protective against cancer as these probiotics help in the normalization of intestinal permeability, produce antimutagenic organic acid, and enhance the immune system of the host (Kechagia *et al.*, 2013).

1.6.4.5 Antioxidant Activity

Probiotics increase the production of antioxidant biomolecules such as polysaccharides, or stimulate the production of antioxidant enzymes such as Superoxide dismutase (Rezaei *et al.*, 2020). They reduce a pro - oxidant state of the body by inhibiting reactive oxygen species, chelating metals, and encourage ascorbate autoxidation (Rezaei *et al.*, 2020).

1.6.4.6 Cardioprotective Effect

Probiotics help maintain normal blood pressure (Kechagia *et al.*, 2013). They also aid in maintaining a normal lipid profile by decreasing Low density Lipoproteins, Very Low density Lipoproteins, and increasing High density Lipoproteins (Mazloom, Yousefinejad and Dabbaghmanesh, 2013).

1.6.4.7 α - Amylase and α - Glucosidase Inhibition by Probiotics

A systematic review indicated that probiotics have positive impact on fasting, postprandial blood glucose levels, glycated hemoglobin, insulin and insulin resistance (Razmpoosh *et al.*, 2016).

1.7 Health Benefits of Camel Milk

Camel milk has also been observed to have antidiabetic, antiproliferative, antioxidant and antihypertensive potential (Mihic *et al.*, 2016; Ali Redha *et al.*, 2022). Its antidiabetic activity is ascertained to the naturally occurring insulin in the milk which resists proteolysis and is easily absorbed by the body. It is also hypothesized that the insulin in camel milk is encapsulated within vesicles that resist gastric proteolysis. The ability of the milk to exert anti diabetic action could be also be due to the other bioactive molecules present in the milk (Malik *et al.*, 2012). The antihypertensive action of the milk is ascribed to the presence of peptides, which upon appropriate hydrolyses bind to the active sites of Angiotensin Converting Enzyme (ACE) (Mudgil *et al.*, 2019). Various studies have been conducted where the Camel milk was fermented using different bacterial strains and their bioactive potential investigated (Tables 1 - 4).

1.8 Digestion using INFOGEST

The gastrointestinal environment is harsh for the survival of bioactive compounds. The acidic nature of the stomach and the alkaline nature of pancreatic secretions coupled with the activity of various enzymes disintegrate/ inactivate the bioactive components in food. Health benefits related to the food can only be observed if the bioactive compound survives the gastrointestinal tract.

As human studies may not be very feasible under all circumstances, a simulation of *in – vitro* gastrointestinal digestion can be performed by the INFOGEST machine. The bioactive food components post an INFOGEST digestion could be analysed for anti-bacterial, anti-hypertensive, anti-cancerous, anti-diabetic and anti-inflammatory properties.

Similar to the physiological digestion of food, any food material placed in an INFOGEST machine undergoes oral, gastric and intestinal digestion. Standard assays of enzymes required in digestion such as but not limited to amylase, pepsin, lipase (both gastric and pancreatic), trypsin and chymotrypsin are used during an INFOGEST digestion to closely mimic normal physiological conditions. Under normal physiological conditions within the human body, in the oral cavity an enzyme commonly referred to as salivary amylase breakdowns carbohydrates. No protein or fat digestion occurs within the mouth. The food is then transferred to the stomach where pepsin helps in the breakdown of proteins to smaller peptide fragments. Trypsin and chymotrypsin also help in the breakdown of peptide chains. Lipases are secreted both in the stomach and by the pancreas and help in the breakdown of fats. Bile secreted by the liver helps in the emulsification of fats (Brodkorb *et al.*, 2019).

During an INFOGEST oral digestion phase (similar to normal physiological conditions), food material placed in the machine is exposed to salivary amylase for 2 min at pH 7. The food bolus then moves towards INFOGEST gastric phase of digestion where the food material is exposed to pepsin and gastric lipase for 2 h at pH 3. Post this phase, an intestinal phase of digestion is initiated by the INFOGEST machine where intestinal secretions like bile salts and pancreatic enzymes act on the food bolus for a period of 2 h at pH 7 (Brodkorb *et al.*, 2019). If the food components show any

bioactive potential post the digestion by the INFOGEST machine, it is likely that the health potential would be exhibited in human models too.

1.9 Hypothesis and Objective of the Study

Fermentation using appropriate strains would result in the formation of various bioactive peptides (Tables 1 - 4). If these peptides could resist the harsh environmental conditions of the gastrointestinal tract, they could exert good health benefits. The ability of the peptide to resist gastrointestinal conditions can be studied using an INFOGEST machine.

Thereby, this project aimed to ferment Camel milk using an appropriate concoction of probiotic (the strain of which has not been investigated before) and starter culture. Secondly, the project investigated the probiotic survivability in fermented Camel milk under *in - vitro* gastrointestinal digestion (INFOGEST). It also examined the biological activities of the bio accessible compounds in the fermented Camel milk after *in - vitro* digestion. A comparative analysis with fermented Bovine milk was also performed.

Table 1: Previous Studies Conducted on Fermented Camel Milk Representing its Antioxidant Potential

Microorganism	Variable	ABTS	DPPH	Reference
Streptococcus thermophilus LMD - 9 strain	Total Protein	1925 ± 90	-	(El Hatmi *et al.*, 2016) **
Streptococcus thermophilus LMD - 9 strain	>10 kDa	817 ± 60	-	
Streptococcus thermophilus LMD - 9 strain	3 - 10 kDa	525 ± 60	-	
Streptococcus thermophilus LMD - 9 strain	<3 kDa	1371 ± 90	-	
Leuconostoc lactis PTCC 1899	Control	439.19	7.21	(Soleymanzadeh *et al.*, 2019) **
Leuconostoc lactis PTCC 1899	Whole Whey	895.27	8.09	
Leuconostoc lactis PTCC 1899	5 - 10 Kda	2162.16	13.48	
Leuconostoc lactis PTCC 1899	3 - 5 Kda	1807.43	13.71	
Leuconostoc lactis PTCC 1899	< 3 Kda	1875	16.52	
Lb. plantarum DSM2648	Day 0	38.2	1.39	(Ayyash *et al.*, 2018b) *
Lb. plantarum DSM2648	Day 21	39.6	12.2	
Lb. reuteri KX881777	Day 0	48.6	16.0	
Lb. reuteri KX881777	Day 21	60.8	31.4	
Lb. plantarum KX881779	Day 0	47.3	20.2	
Lb. plantarum KX881779	Day 21	71.2	46.4	
Lb. plantarum KX881772 (Lp.K772)	Day 0	40.0	3.5	
Lb. plantarum KX881772 (Lp.K772)	Day 21	51.8	38.4	
Lb. acidophilus DSM9126	Day 21	33.4	3.0	(Ayyash *et al.*, 2018a) *
Lb. acidophilus DSM9126	Day 0	39.8	10.3	
Lactococcus lactis KX881782	Day 21	58.4	31.7	
Lactococcus lactis KX881782	Day 0	54.3	54.1	

* All values for ABTS and DPPH are represented in percentage; ** All values for ABTS and DPPH are in µm Trolox equivalent antioxidant capacity
ABTS: 2,2`-azino - bis(3 - ethylbenzo - thiazoline - 6 - sulphonic acid) , DPPH: 1,1 - diphenyl - 2 - picrylhydrazyl, Lb: *Lactobacillus*

Table 2: Previous Studies Conducted on Fermented Camel Milk Representing its Antihypertensive Potential

Microorganism	Variable	ACE	Reference
Leuconostoc lactis PTCC 1899	Control	-	(Soleymanzadeh *et al.*, 2019) **
Leuconostoc lactis PTCC 1899	Whole Whey	3.47	
Leuconostoc lactis PTCC 1899	5 - 10 Kda	1.73	
Leuconostoc lactis PTCC 1899	3 - 5 Kda	1.78	
Leuconostoc lactis PTCC 1899	< 3 Kda	1.62	
Lb. plantarum DSM2648	Day 0	16.4	(Ayyash *et al.*, 2018b) *
Lb. plantarum DSM2648	Day 21	31.6	
Lb. reuteri KX881777	Day 0	81.8	
Lb. reuteri KX881777	Day 21	86.5	
Lb. plantarum KX881779	Day 0	60.0	
Lb. plantarum KX881779	Day 21	78.9	
Lb. plantarum KX881772 (Lp.K772)	Day 0	38.5	
Lb. plantarum KX881772 (Lp.K772)	Day 21	65.1	
Lb. acidophilus DSM9126	Day 21	36.9	(Ayyash *et al.*, 2018a) *
Lb. acidophilus DSM9126	Day 0	62.7	
Lactococcus lactis KX881782	Day 21	81.4	
Lactococcus lactis KX881782	Day 0	85.2	

* All values for ACE are represented in percentage

** All values for ACE are in mg/mL

ACE: Angiotensin Converting Enzyme, Lb: *Lactobacillus*

Table 3**:** Previous Studies Conducted on Fermented Camel Milk Representing its Anticancer Potential

Microorganism	**Day**	**CACO 2 (%)**	**MCF 7 (%)**	**HELA (%)**	**Reference**
Lb. plantarum DSM2648	0	37.5	41.5	42.1	(Ayyash *et al.*, 2018b)
Lb. plantarum DSM2648	21	39.8	47.9	53.2	
Lb. reuteri KX881777	0	37.5	42.6	47.2	
Lb. reuteri KX881777	21	45.6	55.4	60	
Lb. plantarum KX881779	0	37.8	44.7	50.4	
Lb. plantarum KX881779	21	37.3	46.9	54.6	
Lb. acidophilus DSM9126	0	42.1	37.1	46.4	(Ayyash *et al.*, 2018a)
Lb. acidophilus DSM9126	21	42.5	56.4	60.6	
Lactococcus lactis KX881782	0	86.3	88.3	90	
Lactococcus lactis KX881782	21	92.8	91.5	87.4	

CACO 2: Colon cancer cell line, MCF 7: Breast cancer cell line, HELA: Cervical cancer cells

Table 4: Previous Studies Conducted on Fermented Camel Milk Representing its Ability to Inhibit α - Amylase and α - Glucosidase

Microorganism	**Day**	**α - Amylase (%)**	**α - Glucosidase (%)**	**Reference**
Lb. plantarum DSM2648	0	33.33	25.64	(Ayyash *et al.*, 2018b)
Lb. plantarum DSM2648	21	38.47	31.53	
Lb. reuteri KX881777	0	41.54	25.89	
Lb. reuteri KX881777	21	49.25	29.48	
Lb. plantarum KX881779	0	40.74	25.89	
Lb. plantarum KX881779	21	43.81	41.79	
Lb. plantarum KX881772 (Lp.K772)	0	23.71	31.79	
Lb. plantarum KX881772 (Lp.K772)	21	33.22	33.07	

Lb: *Lactobacillus*

Chapter 2: Materials and Methods

2.1 Sample Collection and Preparation

Camel and Bovine full cream milk powder was purchased from a local market (Alain farms, Alain, United Arab Emirates). Camel and Bovine milks were prepared in 500 mL scotch bottles by dissolving 50 g of powder in 400 mL water. To ensure a homogenous mixture, magnetic stirring was employed. The samples were stored at 4°C overnight for hydration purposes. Pasteurization was performed at 105°C for 5 min, the following day. The sample was then cooled to 45°C in an ice - bath.

2.2 Probiotic Bacterial Culture Preparation

The starter cultures, *Lactobacillus delbrueckii* subsp. *delbrueckii* DSMZ20074 and *Lactobacillus delbrueckii* subsp. *bulgaricus* DSMZ20081 was obtained from Leibniz - Institut DSMZ - Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ, Braunschweig, Germany). The probiotic, *Lactococcus lactis* C47 was stored in de Man, Rogosa, and Sharpe (MRS) broth (Neogen, Heywood, UK) with 50% glycerol at -80°C. The culture was incubated at 37°C for 20 h before use. Two consecutive sub - culturing was performed in MRS broth before adding the culture to the milk so as to reach a final cell concentration of 10^7 CFU/mL.

2.3 Fermentation

The bottles from both Camel and Bovine milk were divided into four sets of treatments *viz*. T1 (control), T2 (milk with commercial two *Lactobacillus* strains) (1:1), T3 (milk with commercial *Lactobacillus* culture and probiotic culture) (1:1) and T4 (milk with probiotic culture). The bottles were stirred for 1 - 2 min with the help of a magnetic stirrer and stored at 42°C until the pH reached to 4.5 - 4.6. They were then placed

under refrigeration conditions (4°C) up to 21 days (Ayyash *et al.*, 2018b). Analysis was conducted only on two days (day 0 and day 21). Three replicates from each treatment were *in - vitro* digested according to the INFOGEST static digestion protocol by Brodkorb *et al.,* (2019).

For the water - soluble extract (WSE) preparation, the pH was adjusted to 4.6 after which centrifugation was performed at 5,000 × g for 20 min at 4°C. The resulting supernatant was filtered through a syringe filter (0.45 - µm) (mixed cellulose esters, EMD Millipore Corp., Billerica, MA) (Ayyash *et al.*, 2018a).

2.4 pH and Titratable Acidity

The pH was measured using a pH meter Start - 3100 pH meter (OHAUS Corporation, NJ, USA) after calibration. Titratable acidity (expressed as Lactic acid percentage) was measured using 0.1 M NaOH and phenolphthalein as an indicator.

2.5 Preparation of Oral, Gastric, and Intestinal Simulatory Fluids Master Mix

For the preparation of 400 mL of simulated digestion fluid, the minimum volume of electrolyte stock solutions needed at a 1.25× concentration are as follows

0.3 M $CaCl_2$ $(H_2O)_2$, 0.5 M KCl, 0.5 M KH_2PO_4, 1 M $NaHCO_3$, 2 M NaCl, 0.15 M $MgCl_2(H2O)_6$, 0.5 M $(NH4)_2CO_3$.

The pH of stock solutions of simulated digestion fluids was adjusted using 1 M NaOH and 1 M HCl (a minimum of ~5 mL). Stock solutions were stored in aliquots at −20°C.

Simulated digestion fluids for the oral (SSF), gastric (SGF), and intestinal (SIF) digestion phases were mixed at a 1.25 times concentration using the electrolyte stock solutions and water. The stock solutions of simulated digestion fluids were used to prepare working solutions of 1x concentration.

Master - mix for SSF, SGF, and SIF were prepared for 12 tubes. Fresh enzyme working solutions of Human salivary α - Amylase, Porcine pepsin, Rabbit gastric extract for gastric lipase, Porcine pancreatin, and Bovine bile were prepared from stock solutions immediately before digestion. All the enzyme working solutions were placed on ice to avoid denaturation.

2.5.1 Oral Digestion

For oral digestion, 3 mL of SSF was added to 3 mL milk sample to reach a final volume of 6 mL. An amylase concentration of 75 U/mL was used. The mixture was then kept in a shaking water bath (Witeg Labortechnik GmbH, Wertheim, Germany) for 10 min at 37°C.

2.5.2 Gastric Digestion

To initiate gastric digestion, another 6 mL of SGF was added to the oral digested milk samples to reach a final volume of 12 mL. Pepsin and gastric lipase (rabbit gastric extract, Lipolytech, Marseille, France) concentration of 2000 and 60 U/mL were used, respectively. The pH was adjusted to 3.0 using 1.0 M HCl. The tubes were then placed at 37°C for 2 hours in a shaking water bath.

2.5.3 Intestinal Digestion

To initiate intestinal digestion, another 12 mL of SIF was added to the 12 mL of gastric digested milk samples so as to reach a final volume of 24 mL. Trypsin and Bile salts were added at concentrations of 100 U/mL and 10 mM at pH 7.0, respectively. A dialysis tubing membrane (10 kDa MWCO, Thermofisher, UK) filled with 25 mL of 0.5 M $NaHCO_3$ was placed within the solution. This separated the bioaccessible (10

kDa - permeable) and non - bioaccessible (10 kDa - non - permeable; excreta) fractions of the digesta (Rodríguez - Roque, Rojas - Graü, Elez - Martínez, and Martín - Belloso, 2013). The samples were incubated at 37°C for 2 hours in a shaking water bath. After completion of the intestinal digestion phase, the dialysis membrane was removed and rinsed with double distilled water.

2.6 Bacterial Enumeration

The total viable numbers of *Lactobacilli* and *Lactococci* were enumerated in duplicate on MRS agar (Neogen, Heywood, UK) and M17 agar (HiMedia, India), respectively. The plates were incubated under anerobic conditions (CO_2: 5.0%, Binder GmbH, Tuttlingen, Germany) at 37°C for 48 and 24 hours, respectively. The enumeration was performed prior to the *in - vitro* digestion and after each digestion step (Ayyash *et al.*, 2018a).

2.7 Degree of Hydrolysis

Degree of hydrolysis was measured using the OPA (o - phthalaldehyde) assay described by Sah *et al.,* (2014) with a minor modification. The OPA reagent was prepared freshly by mixing 25 mL of sodium tetraborate buffer (100 mM; at pH 9.3), 2.5 mL of sodium dodecyl sulphate (20%, w/v), 40 mg of OPA dissolved in 2 mL methanol, and 100 µL of β - mercaptoethanol in 50 mL volumetric flask. The double distilled water was used to top up the volume of the 50 mL flask. In a 96 well plate, 60 µL of each *in - vitro* digested milk sample was mixed with 240 µL of OPA reagent per well. The mixture was incubated at room temperature for 2 min. The absorbance was determined at 340 nm by using a UV - spectrophotometer (Epoch™ Microplate Spectrophotometer). Degree of hydrolysis was calculated using the following equation:

$$\text{Degree of Hydrolysis (\%)} = \frac{h}{h\ tot} \times 100$$

Where, h_{tot} the total number of peptide bonds per protein equivalent which had a value = 7.6 mEq/g protein (Nielsen, Petersen, and Dambmann, 2001), while h is the number of hydrolysed bonds.

Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS - PAGE) was performed as per the procedure stated by Ong and Shah (2009).

2.8 Antioxidant Activity

The 1,1 - diphenyl - 2 - picrylhydrazyl (DPPH) assay was used to evaluate the capability of *in - vitro* digested milk to scavenge free radicals according to the method described by Ayyash *et al.,* (2018a). The DPPH reagent was prepared by dissolving 0.1 mM DPPH in 95% methanol. About 800 µL of the DPPH reagent was then added to 200 µL of milk sample. After vigorous shaking, the mixture was incubated for 30 min at room temperature in the dark. Methanol was employed as a blank. After incubation, absorbance was determined at 517 nm using a UV - spectrophotometer (Epoch™ Microplate Spectrophotometer).

The assessment of radical scavenging activity was also performed using a 2,2´-azino - bis(3 - ethylbenzo - thiazoline - 6 - sulphonic acid) (ABTS•+) assay as per the protocol of Ayyash *et al.,* (2018a). The ABTS stock was prepared by mixing 2.6 mM potassium persulphate and 7.4 mM ABTS in equal quantities. The mixture was stored in the dark for 12 h at room temperature. ABTS reagent was prepared freshly by mixing 1 mL of ABTS stock with 50 - 60 mL buffered methanol and equilibrated at 30°C to reach an absorbance of 0.70 ± 0.02 at 734 nm. Two milliliters of ABTS reagent was added to 20 µL of *in - vitro* digested milk and incubated for 6 min at 30°C. The absorbance was

measured at 734 nm. Radical scavenging activity was determined using the following equation:

$$\text{Scavenging rate \%} = \left(\frac{Abs\ blank - Abs\ sample}{Abs\ blank}\right) \times 100$$

2.9 α - Amylase and α - Glucosidase Inhibition Assay

α - Amylase inhibition activity was determined as per the method described by Ayyash *et al.,* (2018a). α - Amylase (1.0 unit/mL, Sigma) (100 µL) was mixed with 100 µL of *in - vitro* digested milk samples and incubated for 5 min at 37°C. Post the incubation period, 1% corn starch solution (250 µL) dissolved in 20 mM Phosphate buffer solution (PBS) (pH 6.8) was added as a substrate to the existing mixture. The resulting solution was then incubated for 5 min at 37°C after which 200 µL of DNS reagent (1% 3,5 - dinitrosalicylic acid and 12% sodium potassium tartrate in 0.4 M NaOH) was further added to terminate the reaction. The entire mixture was then heated at 100°C for 15 min using a water bath. About 2 mL of distilled water was then added in an ice bath. The absorbance was determined at 540 nm.

α - Glucosidase inhibition assay was also performed (Ayyash *et al.*, 2018a). α - Glucosidase (1 unit/mL) was dissolved in 100 µL of 0.1M PBS (pH 6.8) and mixed with 50 µL of the digested milk sample followed by incubation for 5 min at 37°C. An aliquot (50 µL) of 5 mM pnitrophenyl α - D - glucopyranoside (pNPG) was added to the resulting solution and the mixture incubated for 30 min at 37°C. The reaction was terminated by adding 1 mL of 0.1M Na_2CO_3. The absorbance was measured at 400 nm. A mixture without pNPG (the substrate) was prepared as a blank, and the mixture without the intestinal milk digest was considered as a control. The percentage of α - Glucosidase inhibition was determined using the following equation:

$$\text{Inhibition \%} = \left(1 - \frac{Abs\ sample - Abs\ blank}{Abs\ control}\right) \times 100$$

2.10 ACE - Inhibition

Angiotensin Converting Enzyme (ACE) inhibition activity of *in - vitro* digested milk samples evaluated as per the procedure described by Ayyash *et al.,* (2018a). ACE enzyme (from rabbit lung) and hippurly - histidyl - leucin (HHL) was dissolved in 1 mL of Tris buffer (50 mM, pH 8.3) supplemented with 300 mM NaCl. The assay consisted of 100 µL of 3.0 mM HHL, 100 µL of ACE enzyme (1.25 mU/mL), and 100 µL of the milk sample. The mixture was incubated at 37°C for 30 min in a water bath both prior to and after mixing. To terminate the ACE enzyme activity, glacial acetic acid (200 µL) was added. The mixture was stored at -20ºC. The hippuric acid released because of ACE enzyme action on the HHL was determined using High - Performance Liquid Chromatography (HPLC). An aliquot of 200 µL of the mixture was injected into the HPLC system (ThermoFisher Scientific, Waltham, MA, USA) consisting of a reverse - phase column (C18, 250 - mm length, 4.6 - mm diameter, 5 µm diameter) and a guard column (C18 4 - 3.0 mm). The separation was conducted at room temperature (~22°C) at a flow rate of 0.8 mL/min. The mobile phase was an isocratic system consisting of 12.5% (v/v) acetonitrile in distilled water with adjusted pH to 3.0 using glacial acetic acid. The detection was carried out with a UV - Vis detector set at 228 nm. The control consisted of a 100 µL of buffer instead of the *in - vitro* digested milk sample. The rate of ACE inhibition activity was determined as per the following equation:

$$\text{ACE inhibition \%} = \left(\frac{HA\ control - HA\ sample}{HA\ control}\right) \times 100$$

2.11 Antiproliferative Activity

Antiproliferative activity was assessed against colon (HT - 29) and breast (MDAMB - 231) carcinoma cell lines (Ayyash *et al.*, 2018a). The antiproliferative effect was calculated as follows:

$$\text{Antiproliferative \%} = 100 - \left(\frac{Control\ viability - Treatment\ viability}{Control} \times 100\right)$$

2.12 Statistical Analysis

The statistical analyses were carried out by XLSTAT software (Addinsoft, New York, NY, USA). A One-way ANOVA was performed to investigate the effect of the starter bacteria, and storage time. Means comparisons were performed using Tukey's test ($p < 0.05$). All analysis were conducted in triplicate unless stated otherwise. Principal Component Analysis (PCA) was performed to investigate the relationships between variables and to identify the relationship between the various fermentation treatments conducted on each milk type. The PCA was performed by Beijing Genomics Institute (Shenzhen, China).

Chapter 3: Results and Discussion

3.1 Effect of Fermentation on pH and Titratable Acidity (%)

A closer look at Figure 1A and C shows that the pH of milk (upon refrigerated storage for 21 days) was the lowest when fermentation was performed using SC (*Lactobacilli*) and SC+Pro (*Lactobacilli* + *Lactococci*) when compared to exclusive fermentation by Pro (*Lactococci*) or the control in both Camel and Bovine milk, respectively ($p < 0.05$).

Concerning total acidity (Figure 1 B and D), it was the highest in milk fermented with SC (*Lactobacilli*) and SC+Pro (*Lactobacilli* + *Lactococci*) when compared to exclusive fermentation by Pro (*Lactococci*) or the control ($p < 0.05$). The increase could be attributed to fermentation, with similar observations reported previously (Gomes *et al.*, 2013; Tomovska, Gjorgievski and Makarijoski, 2016). A rather interesting observation in this study was the higher increases in acidity comparative to the decrease in pH, which could perhaps be ascertained to the natural buffering capacity of milk (Attia, Kherouatou and Dhouib, 2001). Milks have high buffering capacity as it naturally contains various organic acids like propionic, lactic and carbonic acids. Their dissociation under appropriate conditions gives rise to anions like propionate, lactate and carbonate. On the other hand, milk also contains cations like calcium, magnesium and phosphorus. They neutralize the anions and hence give milk its natural buffering capacity (Salaün, Mietton and Gaucheron, 2005). The Whey and caseinate proteins inherent to the milk also play a role as buffers (Salaün, Mietton and Gaucheron, 2005).

pH and titratable acidity are both parameters used to assess the acidic nature of foods (Nielsen, 2005). However, a subtle difference does exist between them (Nielsen,

2005). They are important parameters tested in the milk industry to produce fermented products like yogurt and kefir (Tomovska *et al.*, 2016).

In milk, lactose, a naturally occurring sugar, acts as a substrate for fermentation (Tomovska *et al.*, 2016). The resultant products of the process, i.e., organic acids like propionic, acetic acid, and lactic acid, majorly influence the fermented products' acidity (Rakhmanova, Khan and Shah, 2018). Analyzing the pH/ acidity during fermentation is essential as this may affect the survival rates of the inoculated probiotic bacteria, which may eventually affect the product's therapeutic and organoleptic properties (Tian *et al.*, 2017; Neffe-Skocińska *et al.*, 2018). On the other hand, titratable acidity is a more beneficial measure to better understand the product's flavor (Nielsen, 2005).

In a previous study, the fermentation of milk at 30°C by *Lactobacillus delbrueckii* ssp. bulgaricus for 24 hours resulted in a milk pH of 4.25 - 4.40 (depending on strain type) (Gil-Rodríguez and Beresford, 2019). Fermentation at the same parameters by *Lactococcus lactis* resulted in a pH of 4.25 - 4.28 (Gil-Rodríguez and Beresford, 2019). Similarly, fermentation by *Streptococcus thermophilus* and *Lactobacillus delbruescii* ssp. bulgaricus, at 40°C, resulted in a pH drop from 6.67 to 4.19 within 15 days of milk storage (Tomovska *et al.*, 2016). In another study, milk fermentation by a strain mix composed of *Streptococcus salivarius* subsp. thermophilus and *Lactococcus delbrueckii* subsp. bulgaricus for 28 days at 7°C resulted in a pH change from 4.24 to 4.13, respectively (Gomes *et al.*, 2013).

The subtle differences between our study and the reported literature are understandable. The acidity of milk has been observed to depend on various factors

like milk protein content and bacterial growth besides storage time and temperature (Schmidt *et al.*, 1996; Căpriţă, Căpriţă and Creţescu, 2014; M'hamdi *et al.*, 2018).

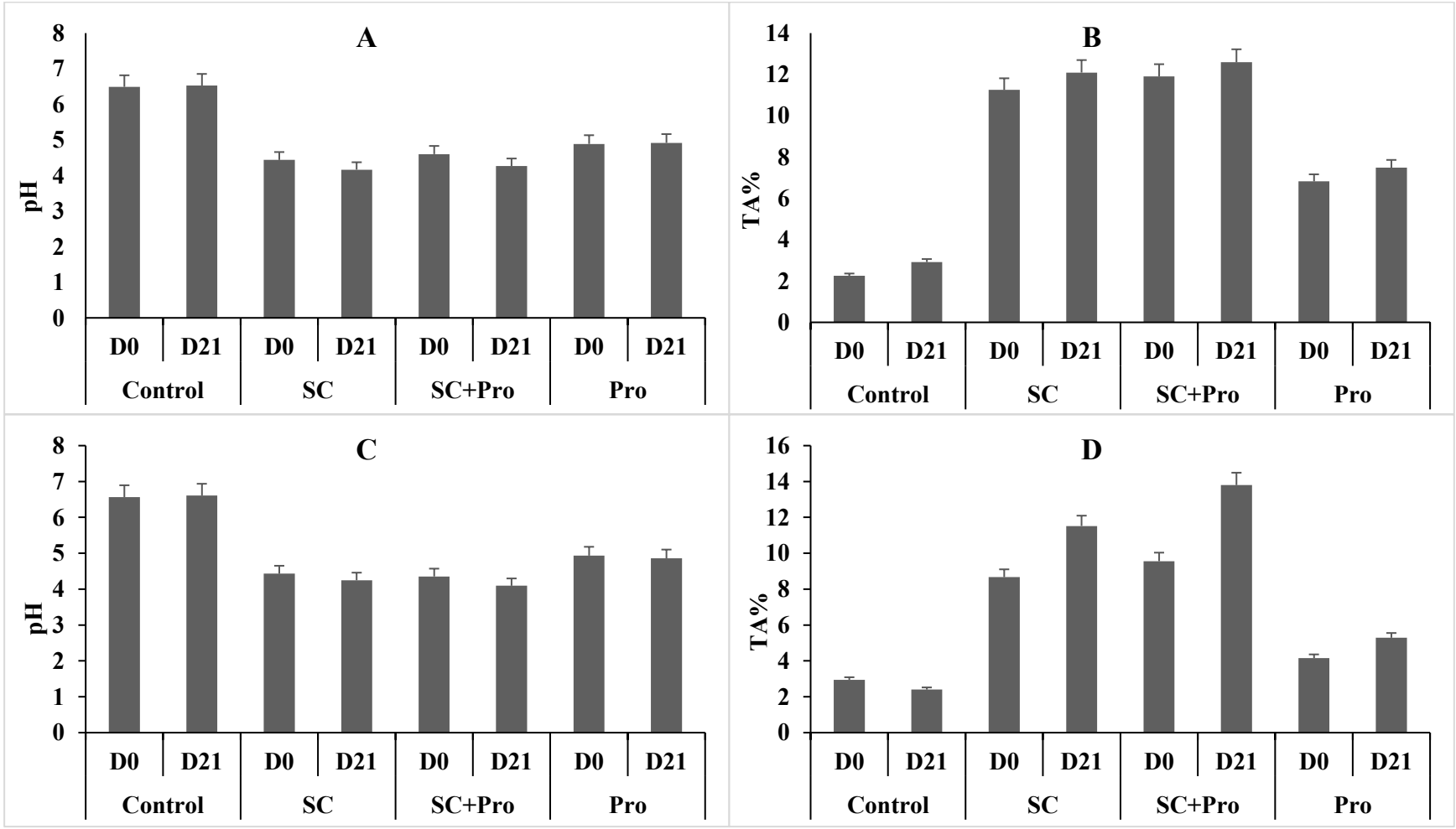

Figure 1: PH and TA values of fermented camel (a and b) and bovine (c and d) milk. SC: *Lactobacillus delbrueckii* subsp. *delbrueckii* and *Lactobacillus delbrueckii* subsp. *bulgaricus* Pro: *Lactococcus lactis.* Bars are mean ±SD of n=3

3.2 Effect of Fermentation on Bacterial Count (log_{10} CFU/mL)

As seen in Table 5, after gastric digestion, the bacterial populations decreased by 0.9 - 3.3, 2.3 - 4.3 log_{10} CFU/mL in Camel and Bovine milk, respectively ($p < 0.05$). In comparison, intestinal digestion decreased the microbial populations by 2.2 - 3.6, 3.1 - 4.6 log_{10} CFU/mL, respectively. The length of the fermentation period had minimal effect on survival of the microorganisms. A previous study on Camel milk LAB isolates observed that acidic and bile conditions decreased the microbial counts ranging from 0.29 to 6.78 log_{10} CFU/ mL, respectively (Abushelaibi *et al.*, 2017). The difference in the decrease of the bacterial populations compared to this study could be attributed to the difference in strains and the variance in milk composition. Although both the studies used Camel milk, the milk composition (in terms of fat and protein) has been observed to vary based on variables like the season, stage of lactation, location, and parity number (Babiker and El-Zubeir, 2014; Elhassan *et al.*, 2015; Ereifej *et al.*, 2011; Haddadin *et al.*, 2008; Zhang *et al.*, 2005).

An interesting observation in this study was that the limited decrease in viable counts posts a gastric/ intestinal digestion treatment in Camel milk compared to Bovine milk. The finding could be due to the Whey particles in Camel milk which have been observed to offer better protection than Bovine milk in a previous study (Ahmad, Mudgil and Maqsood, 2019). Camel milk has also been observed to have different compositions and concentrations of 'protective proteins' compared to milk from other ruminants (Kappeler, 1998).

Another observation in this study was that the viable numbers of Pro (*Lactococci*) decreased more in the presence of SC (*Lactobacilli*) after digestion in both milk types. This could be because the presence of SC (*Lactobacilli*) alongside Pro (*Lactococci*)

resulted in a competition of limited resources in an environment that is already rather unfavorable (due to the gastric and intestinal digestion) (Terpou *et al.*, 2019).

Overall, the survival of Pro (*Lactococci*) was the highest after both gastric and intestinal digestion compared to SC (*Lactobacilli*) alone. The high acid tolerance of this species has been observed previously (Abushelaibi *et al.*, 2017). *Lactococcus* strains previously have been observed to not survive very well under *in - vitro* human gastric juice conditions; however, in the presence of *in - vitro* duodenal juices, they have been observed to resurrect themselves (Faye *et al.*, 2012).

Probiotics have proven to be a boon for the food industry, with vegetable/ fruit/ dairy probiotic - based products available in the market (Song, Ibrahim and Hayek, 2012). A significant increase in interest pertaining to probiotics could also be attributed to their multi - faceted health benefits (Isolauri, Kirjavainen and Salminen, 2002). However, they need to survive the harsh gastrointestinal conditions (Han *et al.*, 2021). The acidic gastric juices, bile, pancreatic enzymes all hurdles probiotic survival (Han *et al.*, 2021). To exert a beneficial effect, probiotic products should contain > 10^6 CFU/g (CFU/mL) of the microorganism (Neffe-Skocińska *et al.*, 2018). In this study, bacterial populations of Pro (*Lactococci*) solely met this criterion in both Camel and Bovine milk post gastric and intestinal digestion. Perhaps, the microencapsulation of the probiotic would further improve their survival (Ahmad, Mudgil and Maqsood, 2019).

Factors like acidic/ basic nature of the food matrix, oxygen availability in the food product, presence of other competing LAB, besides the ability to resist any toxic metabolites produced by the other bacteria in the food matrix in general, have been observed to affect the survival of probiotics in previous studies (Terpou *et al.*, 2019).

Table 5: The Bacterial Population (Log_{10} CFU/mL) in Fermented Camel and Bovine milk During *in - vitro* Digestion

	D0				D21			
	BD	Oral	Gastric	Intestine	BD	Oral	Gastric	Intestine
Camel								
Control	ND[1]	ND	ND	ND	ND	ND	ND	ND
SC[2]	6.56±0.11a	6.59±0.21a	5.58±0.02b	4.33±0.14c	8.09±0.08a	6.87±0.15ab	5.64±0.10b	4.54±0.19c
Pro[3]	7.43±0.14a	6.55±0.16a	5.78±0.09b	5.53±0.06b	8.75±0.09a	7.72±0.04a	6.44±0.01b	6.27±0.06b
SC+Pro	7.50±0.44[4a]	6.72±0.07a	5.41±0.06b	4.69±0.09c	8.41±0.39a	7.32±0.07ab	5.82±0.03b	5.01±0.16b
	8.93±0.12[5a]	6.32±0.12b	5.54±0.16c	5.30±0.18c	8.81±0.04a	7.42±0.05ab	5.97±0.02b	5.18±0.02b
Bovine								
Control	ND	ND	ND	ND	ND	ND	ND	ND
SC	7.81±0.19a	6.90±0.18ab	3.65±0.92b	3.69±0.09b	8.00±0.02a	6.86±0.05b	4.23±0.21c	4.09±0.05c
Pro	8.67±0.05a	7.69±0.04a	6.29±0.08b	5.52±0.08b	8.34±0.20a	7.67±0.06a	6.22±0.05b	6.12±0.17b
SC+Pro	8.08±0.21a	6.92±0.06b	5.19±0.16c	3.75±0.18d	8.77±0.02a	6.80±0.15ab	4.38±0.01b	4.15±0.19b
	8.81±0.14a	7.18±0.06ab	5.14±0.03b	4.65±0.09c	8.78±0.05a	6.82±0.05ab	4.56±0.14b	4.42±0.13b

BD: before digestion and after fermentation
1 ND: not detected (< 1 Log CFU/mL)
2 SC: starter culture enumerated on MRS (*Lactobacilli*)
3 Pro: probiotic bacteria enumerated on M - 17 (*Lactococci*)
4,5 SC + Pro: starter culture (*Lactobacilli*) and probiotic (*Lactococci*)

3.3 Effect of Fermentation on the Degree of Hydrolysis (%)

Comparing Camel and Bovine milk, their WSE did not have significant differences in terms of OPA absorption (Figure 2 B and D). However, on the last day of storage, the OPA absorbance was slightly higher in Bovine milk for all the three fractions (WSE, Excreta, Bio accessible fraction) in all fermented milk types. An increase in free amino acid content within 4 hours of fermentation of Camel milk by *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. bulgaricus has been reported previously (Abu-Tarboush, 1996). In terms of the degree of proteolysis, higher resistance to proteolysis in cow milk compared to Camel milk has also been reported (Abu-Tarboush, 1996). The most significant OPA absorbance in Camel and Bovine milk was between 0.7 - 0.8 and 0.6 - 0.7, respectively, in the WSE. Fermentation of Camel milk using *Lactobacillus plantarum* and *Lactobacillus paraplantarum* was observed to increase the OPA absorbance while using *Leuconostoc lactis*, *Enterococcus faecium*, and *Lactobacillus paracasei* resulted in decreasing the OPA absorbance (Soleymanzadeh, Mirdamadi and Kianirad, 2016). In Bovine milk, fermentation using *Leuconostoc lactis*, *Enterococcus faecium*, *Lactobacillus plantarum*, and *Lactobacillus gasseri* enhanced protein hydrolysis (Soleymanzadeh, Mirdamadi and Kianirad, 2016). Using Alcalase, Bromelain, and Papain for Camel milk hydrolysis resulted in a 15.5, 23.8, 39.6% degree of hydrolysis, respectively (Al-Shamsi *et al.*, 2018).

Comparing the different fractions, a usual trend of higher OPA absorbance in the WSE fractions compared to the bio - accessible fractions was observed in both Camel and Bovine milk ($p < 0.05$). This observation could be ascribed to two factors: the decreased protein concentrations in the bio accessible fraction compared to the WSE, and secondly to the limiting nature of the dialysis membrane.

In terms of the treatment with the starter culture and the probiotic bacteria, generally treatment with SC or a combination of SC + Pro enhanced OPA absorption and degree of hydrolysis compared to the usage of a probiotic as a sole agent.

The degree of hydrolysis is defined as the proportion of cleaved peptides upon protein hydrolysis. Fermentation is typically accompanied by proteolysis, with LAB being the primary vehicle carrying this process (Liu *et al.*, 2010). The resulting peptide fractions, or in other words, the extent of proteolysis, would determine the food taste, texture, biological activity and overall consistency (Picon *et al.*, 2010; Chen *et al.*, 2012). In previous studies, the peptides formed due to fermentation have been associated with antihypertensive, anticancer, antioxidant, and antidiabetic properties (Ayyash *et al.*, 2018a).

Concerning the SDS - PAGE (Figure 3), it was observed that all essential proteins in Camel and Bovine milk were degraded after treatment on 0 day and 21 days storage except β - lactoglobulin (18.4 kD). In Lane M1, the ladder of specific proteins lactoferrin (80 kD), Bovine serum albumin (66.4 kD), α - casein (25 kD), β - casein (24 kD), κ - casein (19 kD), β - lactoglobulin (18.4 kD), and α - lactalbumin (14.2 kD) are observable. A slight difference between the protein bands of the two milks fermented using Pro solely is also seen.

The degree of hydrolysis a protein segment in a food matrix goes would depend on its type. A liquid product has shown lower resistance than a semi - solid product (Hernández-Olivas *et al.*, 2020).

The total casein, Whey, α_{S1} - casein, α_{S2} - casein, β - casein, κ - casein, β - lactoglobulin, α - lactalbumin in Camel milk ranges from 22.1 - 26.0, 5.9 - 8.1, 73.27

- 76.24, 4.9 - 5.7, 2.1 - 2.5, 14.4 - 16.9, 0.8 - 0.9, <0.5 and 0.8 - 3.5 gL^{-1}, respectively (Roy *et al.*, 2020). In comparison, the protein profile for other cattle ranges from 24.6 - 28.0, 5.5 - 7.0, 8.0 - 10.7, 2.8 - 3.4, 8.6 - 9.3, 2.3 - 3.3, 3.2 - 3.3 and 1.2 - 1.3, respectively (Roy *et al.*, 2020).

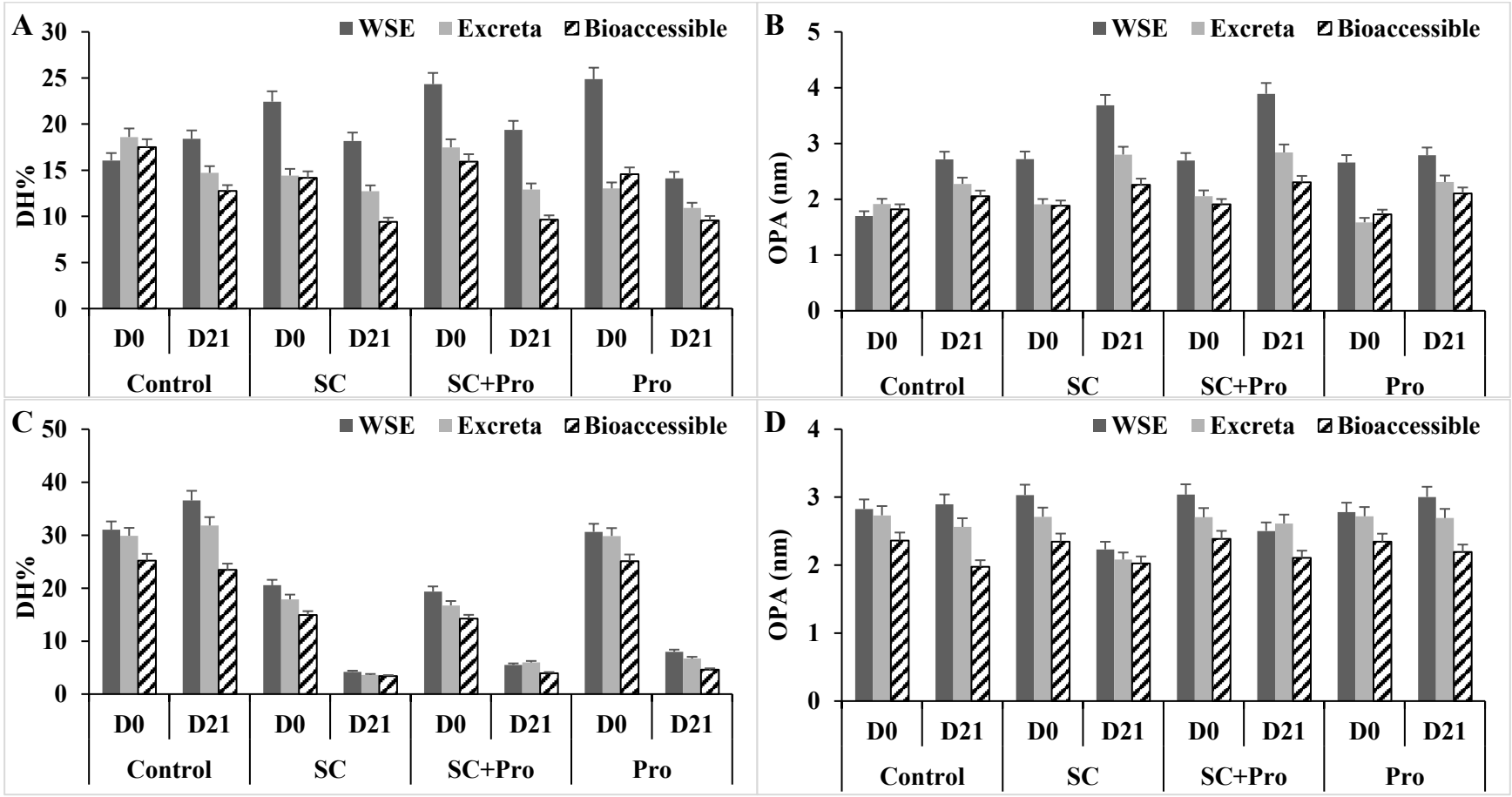

Figure 2: Degree of hydrolysis and proteolysis in fermented camel (a and b) and bovine (c and d) milk. SC: *Lactobacillus delbrueckii* subsp. *delbrueckii* and *Lactobacillus delbrueckii* subsp. *bulgaricus* Pro: *Lactococcus lactis* Bars are mean ±SD of n=3

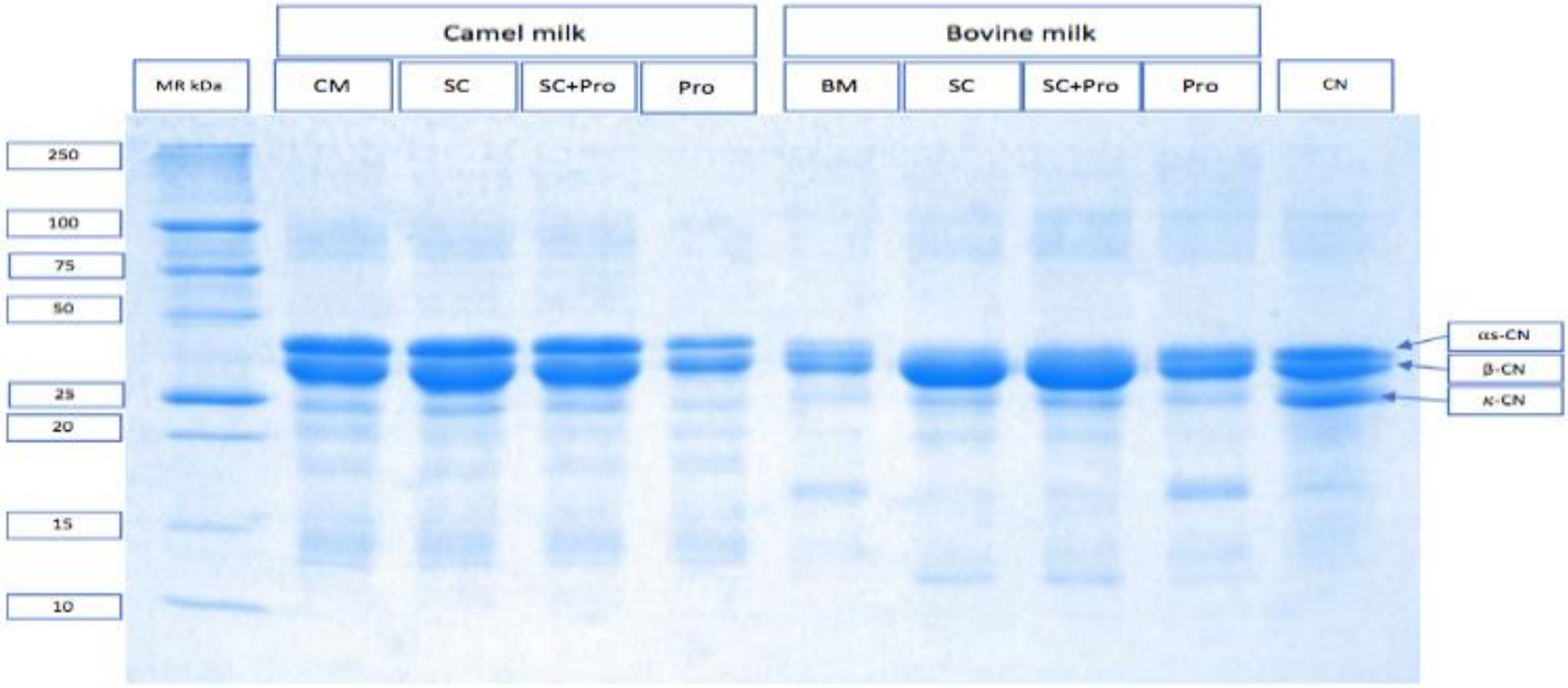

Figure 3: SDS – PAGE at day 1 after complete fermentation. SC: *Lactobacillus delbrueckii* subsp. *delbrueckii* and *Lactobacillus delbrueckii* subsp. *bulgaricus* Pro: *Lactococcus lactis*. Bars are mean ±SD of n=3

3.4 Effect of Fermentation on Antioxidant Activity (Determined by ABTS and DPPH assay)

The antioxidant capacity of fermented Camel milk was evaluated in this work using the ABTS and DPPH assays (Sujarwo and Keim, 2019). It was observed in the DPPH assay (Figure 4 B and D) that all fermentation treatments of Camel milk had higher scavenging activity as compared to its Bovine counterpart ($p < 0.05$). In comparison, a previous study indicated the ABTS scavenging capacity of fermented Bovine and Camel milks to be 20 - 30% and >30% up to 70%, respectively (Ayyash *et al.*, 2018b). A similar observation was made in another study that fermented the milk using *Lactobacillus rhamnosus* PTCC 1637 (Moslehishad *et al.*, 2013). The difference could probably be attributed to the variation in antioxidant amino acid residues in the milks and due to antioxidant substrates like α - lactalbumin and β - lactoglobulin formed as a direct result of proteolysis (Salami *et al.*, 2010). It could also be probably attributed to the higher polyphenol, Vitamin C, and Flavonoid content in Camel milk (27.53 mg/L) compared to Bovine milk (7500 µg/L) (Graulet, 2014; Bouhaddaoui *et al.*, 2019).

Comparing the different fragments of the intestinal digesta, the bio accessible fraction, excreta in Camel and Bovine milk showed a more excellent ABTS scavenging activity compared to the WSE ($p < 0.05$) (Figure 4 A and C). In the previous section, a lower amount of protein in the bio accessible fraction was observed. Thereby the expectation was a lower scavenging activity of this fraction. However, it has been proven otherwise. In other words, it is possible to stipulate from the current observation that the extent of protein hydrolyses matters in determining antioxidant activity. Similar

observations were reported in previous studies (Salami *et al.*, 2010; Moslehishad *et al.*, 2013).

The antioxidant activity of camel whole casein was 3.75 TEAC (μM) while its 5 KDa retentate, 5 KDa permeate and 3 KDa permeate hydrolysates resulted in antioxidant activity of 3.75, 8.23, 7.17, 11.73 and 6.64 TEAC (μM), respectively (Salami *et al.*, 2011). Meanwhile, the antioxidant activity of whole camel β – casein, its 5 KDa retentate, 5 KDa permeate and 3 KDa permeate was 7.30, 17.64, 14.66 and 12.41 TEAC (μM), respectively (Salami *et al.*, 2011).

On the other hand, one study also reported no significant correlation between the extent of hydrolysis and DPPH scavenging activity (El-Sayed, Awad and Abou-Soliman, 2021). The variation in the observation of results can be explained. The antioxidant capacity of the proteins/ peptides depends on the physical structure it has attained post hydrolysis, as the accessibility of scavenging amino acids within the matrix would vary (Dugardin *et al.*, 2020).

Natural cell metabolism in the body generates free radicles. An accumulation of these free radicles puts the body in a state of oxidative stress (Pham-Huy, He and Pham-Huy, 2008). Such a state has been associated with various degenerative diseases like cancer, auto - immune disorders besides aging, and cataract formation (Pham-Huy, He and Pham-Huy, 2008). Probiotics have been observed to exert antioxidant effects previously through a wide range of mechanisms (Mishra *et al.*, 2015).

With respect to the impact of storage, prolonging the storage period was observed to enhance ABTS scavenging activity in the WSE fraction of Camel milk fermented using SC + Pro and sole Pro, respectively. No significant effect was observed on the

excreta and the bio - accessible fraction. In a previous study, the DPPH scavenging activity of Camel milk fermented with four individual strains of *Lactobacillus* was observed to be the highest after day 14 of cold storage, with greater activity compared to commercial starter culture (El-Sayed, Awad and Abou-Soliman, 2021). In another study, DPPH activity of fermented Camel milk increased up to 21 days of storage, while in Bovine milk, an increase up to 14 days was followed by a decrease (Ayyash *et al.*, 2018b).

Comparing the fermentation treatments, treatment with SC and SC + Pro resulted in greater ABTS and DPPH scavenging activity than the control or the Pro alone (Figure 4). The enhanced proteolysis observed in the form of a higher OPA in the previous section could be a possible explanation (Elias, Kellerby and Decker, 2008). Thus, a combination fermentation treatment consisting of both SC and Pro in Camel milk is recommended as the best treatment to improve its antioxidant capacity (compared to the treatments studied in this paper).

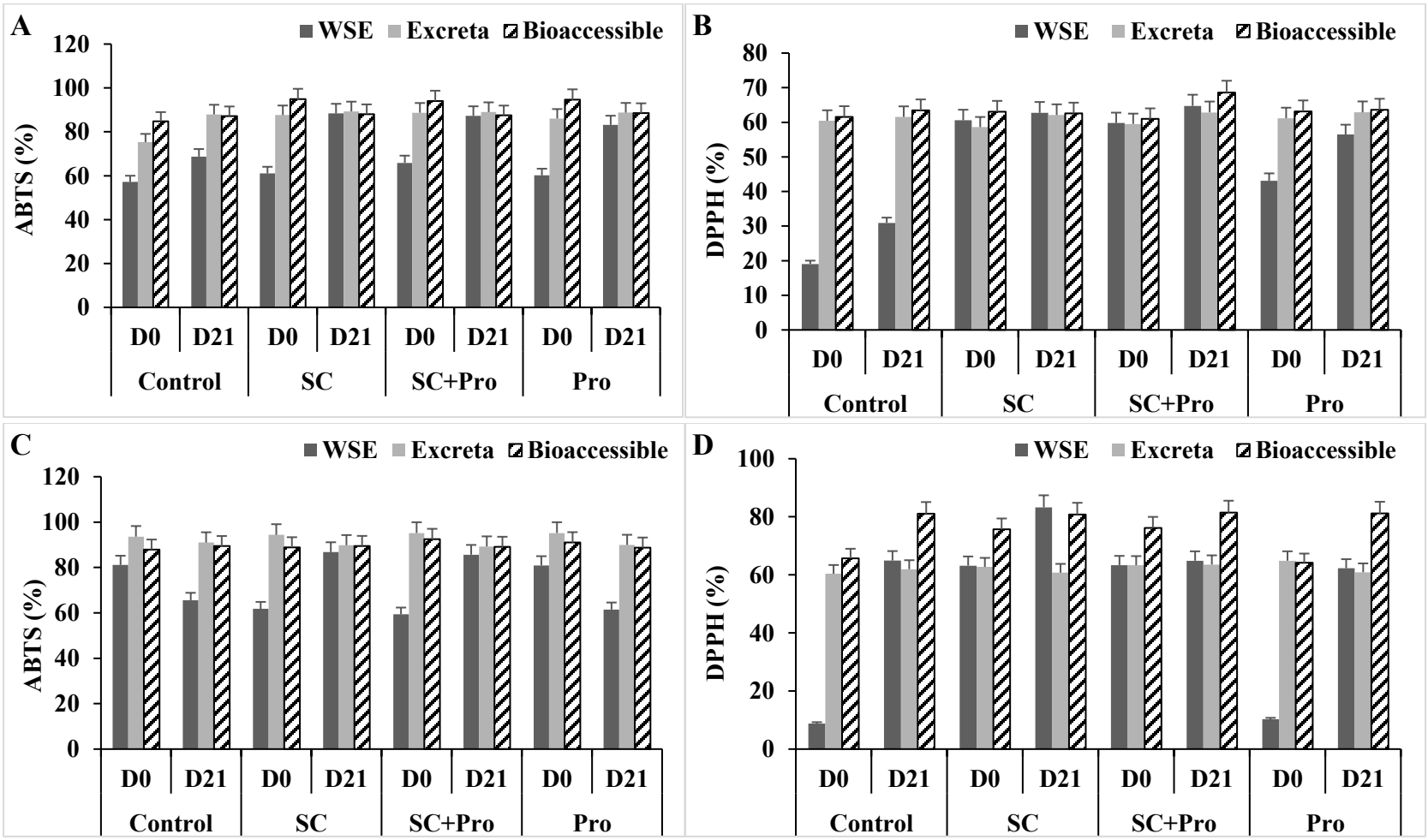

Figure 4: Free radical scavenging activities by ABTS and DPPH of fermented camel (a and b) and bovine (c and d) milk. SC: *Lactobacillus delbrueckii* subsp. *delbrueckii* and *Lactobacillus delbrueckii* subsp. *bulgaricus* Pro: *Lactococcus lactis*. Bars are mean ±SD of n=3

3.5 Effect of Fermentation on the Inhibition of α - Amylase and α - Glucosidase

In this study, comparing the different digested fractions, it was observed that the bio accessible fraction in Camel and Bovine milk had greater α - Amylase inhibiting activity both on the first and last day of storage for all treatments (Figure 5 A, B, C, D). The protein content (in the previous section) was the lowest for the bio accessible fragment. Thereby, it was expected that this fragment would have the lowest α - Amylase inhibiting activity. Multiple previous studies on Camel milk have associated their inherent proteins with antidiabetic properties (Mudgil *et al.*, 2018; Ayyash *et al.*, 2020; Baba *et al.*, 2021). The extent to which a protein is hydrolyzed impacted α - Amylase and α - Glucosidase inhibition as per our results which is in accordance with a previous study, where it was observed that hydrolyzing the Camel milk protein into smaller peptides using Alcalane and bromelain was observed to mildly increase inhibition towards α - Amylase (Mudgil *et al.*, 2018). However, a fine line needs to be drawn regarding the degree of hydrolysis as excessive hydrolysis may also prove futile concerning the antidiabetic effect (Mudgil *et al.*, 2018).

A quarter of the Gulf Cooperation Council (GCC) population is diabetic (Meo, Usmani and Qalbani, 2017). Diabetes is a metabolic condition characterized by insulin resistance or a reduction in insulin secretion, resulting in a rise in blood sugar levels. The enzymes α - Amylase and α - Glucosidase hydrolyze oligosaccharides at the non - reducing ends, thereby releasing the bound glucose (Grom *et al.*, 2020). The released glucose eventually gets into the bloodstream and increases serum glucose levels (Grom *et al.*, 2020). Inhibiting these enzymes slows the breakdown of the oligosaccharides, thereby reducing the abrupt postprandial increase in blood glucose levels (Balisteiro *et al.*, 2017).

With regards to the impact of storage, the inhibition effect on α - Amylase and α - Glucosidase for WSE increased towards the end of storage in Camel and Bovine milks ($p < 0.05$). However, this same trend was not observed for the bio accessible fragment. The overall inhibition rate of α - Amylase and α - Glucosidase in the bio accessible fraction was 48.2 - 77.6% and 50.6 - 88.6% in Camel milk (based on the treatment given). While in Bovine milk, the inhibition was by 45.0 - 78.0% and 33.3 - 89.0%, respectively. A 30 - 40% α - Glucosidase inhibition rate was recorded for Camel and Bovine milk, which were made to undergo fermentation using *Lactococcus lactis* in a previous study (Ayyash *et al.*, 2018b). It is evident that the inhibition activity is dependent on the type of strain used for the fermentation process. Further research is needed to test different variety of probiotic strains with regards to their ability to inhibit these enzymes (α - Amylase and α - Glucosidase). An additional observation was that in α - Glucosidase inhibition, the inhibition of the enzyme by the excreta was comparatively similar to that of the bio accessible fragment in both Camel and Bovine milks.

Comparing the two milks, the WSE of fermented Camel milk resulted in greater inhibition of α - Amylase when compared to its Bovine counterpart. This is in accordance with the study conducted by Shori and Baba (2014), who fermented Camel and Bovine milk using *Lactobacillus acidophilus*, *L. bulgaricus*, *L. casei*, *Bifidobacterium bifidus*, and *Streptococcus thermophilus*. However, it does contradict the findings of another study on Camel milk fermented with *Lactobacillus reuteri* and *Lactobacillus plantarum*, where it was observed that α - Amylase inhibitions of Camel milk were lower than that of Bovine milk (Ayyash *et al.*, 2018b).

Camels are commonly reared in the Arab world due to their ability to survive the harsh weather conditions. The regional estimated rise in diabetes is by 96.2% by 2035 (Abuyassin and Laher, 2016). Thereby, appropriately fermented Camel milk, if marketed well, may help manage the numbers.

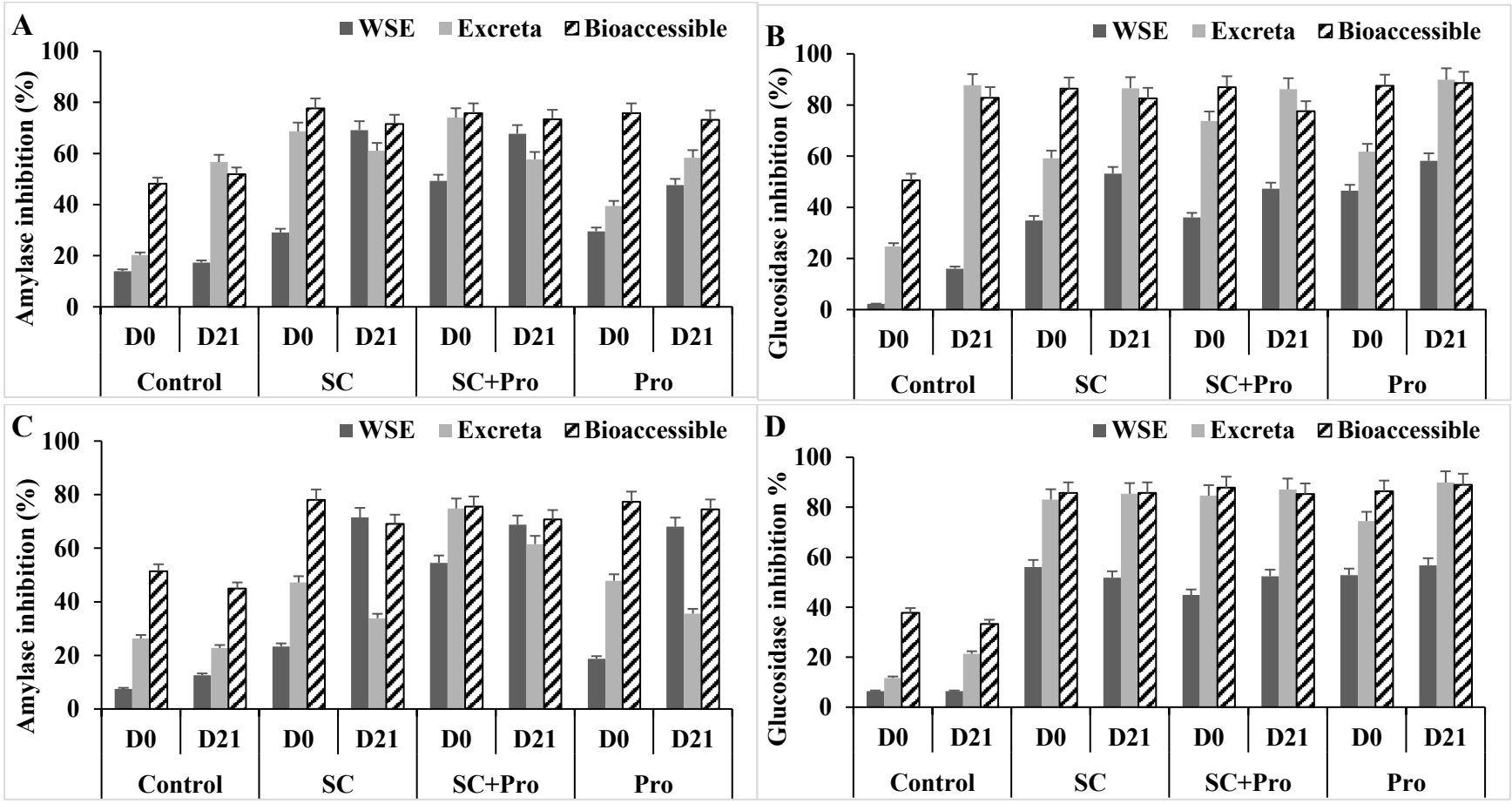

Figure 5: Amylase and glucosidase inhibition in fermented camel (a and b) and bovine (c and d) milk. SC: *Lactobacillus delbrueckii* subsp. *delbrueckii* and *Lactobacillus delbrueckii* subsp. *bulgaricus* Pro: *Lactococcus lactis.* Bars are mean ±SD of n=3

3.6 Effect of Fermentation on ACE - Inhibition Activity

In this study, fermented Camel and Bovine milk (Figure 6 A and B) inhibited ACE during the study storage period by 64 - 86% and 68 - 85%, respectively. Camel milk fermented by *Lactobacillus* spp. had an ACE inhibition rate >80%, while it was <50% in Bovine milk (Ayyash *et al.*, 2018b). A similar observation has been made in more previous studies (Moslehishad *et al.*, 2013; Alhaj *et al.*, 2018). The greater proline content in Camel milk casein compared to Bovine is a possible explanation (El-Salam and El-Shibiny, 2013). Proline rich oligopeptides exert an antihypertensive effect by interacting with specific tissue receptors associated with smooth muscle relaxation, increasing calcium mobilization and nitric oxide production (vasodilator) (Morais *et al.*, 2013).

About 29.5, 27.6% of the population in the Arab world and Sub - Saharan Africa have been reported to suffer from Hypertension (Tailakh *et al.*, 2014). The ACE catalyzes the conversion of Angiotensin I to Angiotensin II, which increases hypertension via fluid retention and noradrenaline release (Fleming, 2006). It also inactivates Bradykinin - a blood vessel dilator (Lumb, 2017). ACE inhibitors exert their antihypertensive effect by preventing the conversion of Angiotensin I to Angiotensin II and the degradation of Bradykinin (Lumb, 2017).

Concerning the effect of storage, in both Camel and Bovine milks, an increase in ACE inhibition of the bio accessible fraction was observed for all fermentation treatments ($p < 0.05$). An increase in ACE inhibition upon cold storage has also been observed previously (Nielsen *et al.*, 2009).

Comparing treatments, the bio - accessible segment of SC or SC + Pro fermented Camel milk had the greatest ACE inhibition effect. Only on the first day of the experiment, the bio - accessibility of SC and SC + Pro fermented Bovine milk was comparable to that of Camel milk. It is evident that adding a Probiotic to the ageing milk resulted in a synergistic effect on ACE activity. The bio accessible fraction was the one with the most negligible molecular weight. Smaller subsections of proteins, i.e., tripeptides such as isoleucine - proline - proline and valine - proline - proline, have been observed to exhibit ACE activities (Rutella *et al.*, 2016). One more study indication a fraction of Camel milk casein to exert the greatest ACE inhibition (Rahimi *et al.*, 2016). An active peptide from Camel milk, MVPYPQR had an ACE inhibition effect of IC_{50} = 30 μm. It exerted its influence due to a hydrogen bond between Glutinine 162 (S'1 pocket of ACE) and Arginine in the C terminal, which distorted the Zn^{2+} tetrahedral geometry of the enzyme (Soleymanzadeh *et al.*, 2019).

The peptides of Camel milk casein hydrolyzed using Chymotrypsin, Pepsin or Trypsin showed ACE inhibitory activity of 80 – 115, 20 – 69, 150 – 300 IC_{50} μg/ml, respectively (based on peptide size) in the retentate. Meanwhile, the ACE activity of the permeate was 95, 23 and 600 IC_{50} μg/ml, respectively (Salami *et al.*, 2011). Hydrolysis of sole camel β - casein by Chymotrypsin, Pepsin or Trypsin resulted in an ACE inhibiting activity ranging from 80 – 103, 23 – 46, 46 – 69 IC_{50} μg/ml in the retentate, respectively. Meanwhile in the permeate the ACE inhibiting activity was observed to be 46, 87 and 94 IC_{50} μg/ml, respectively (Salami *et al.*, 2011).

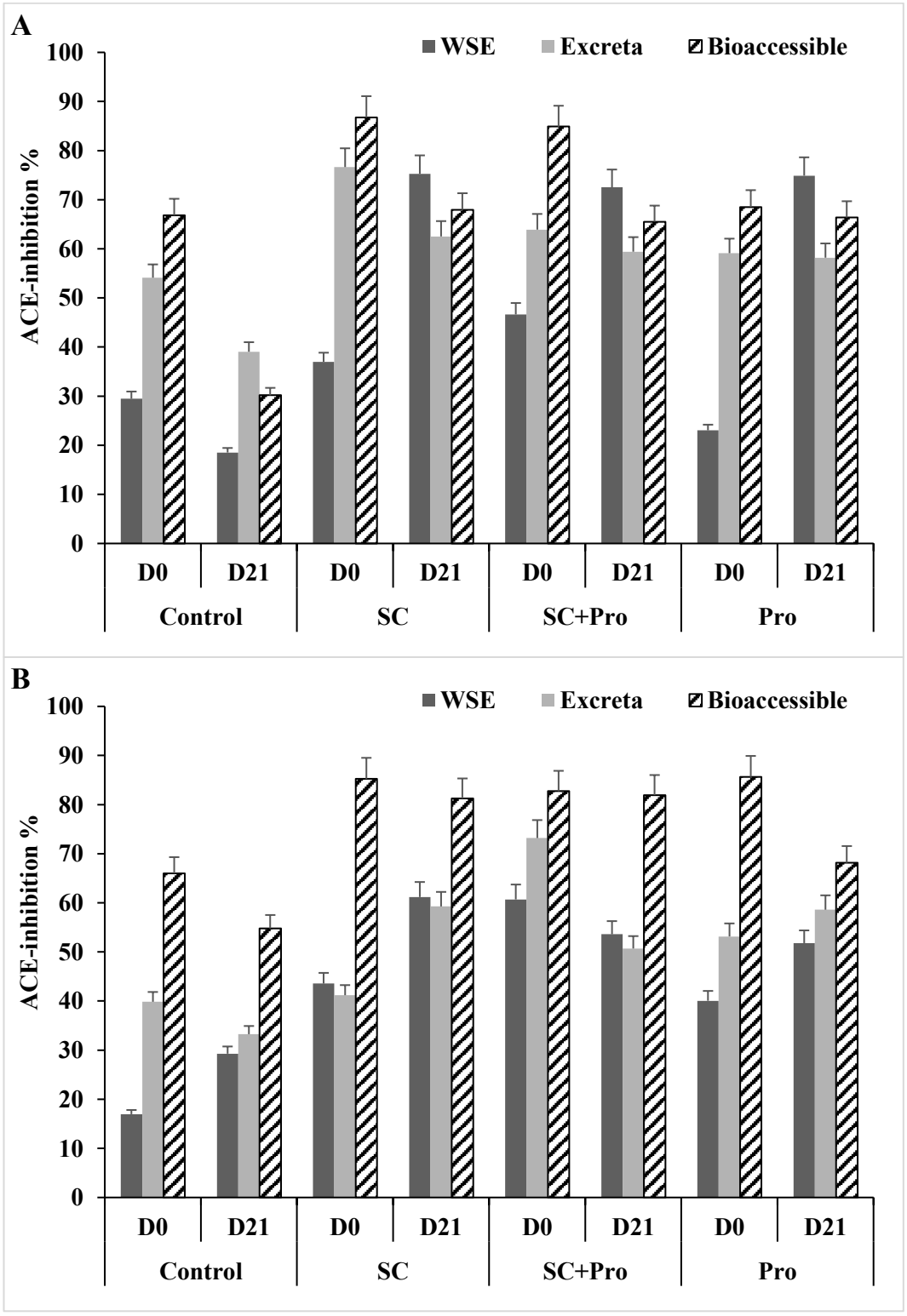

Figure 6: ACE - inhibitions in fermented camel (a) and bovine (b) milk. SC: *Lactobacillus delbrueckii* subsp. *delbrueckii* and *Lactobacillus delbrueckii* subsp. *bulgaricus* Pro: *Lactococcus lactis*. Bars are mean ±SD of n=3

3.7 Effect of Fermentation on Antiproliferative Activity (MDA - MB and HT - 29)

Fermented Camel and Bovine milk (Figure 7 A and B) exhibited an antiproliferative activity of 2.5 - 11.7% and 1.5 - 4.0% in colon cancer cell lines, respectively. Comparing the two milks, the bio accessible fraction of fermented Camel milk had a greater antiproliferation effect than fermented Bovine milk ($p < 0.05$). In a previous study, the WSE of Camel milk fermented by *Lactococcus lactis* inhibited Caco - 2, MCF - 7, and HELA cell lines compared to its fermented Bovine counterpart (Ayyash *et al.*, 2018a). The difference in the matrix of the two milks and the variation in the concentrations of the bioactive peptides are possible explanations for the observed variance between the milks (Pessione and Cirrincione, 2016; Ayyash *et al.*, 2021b).

As of 2020, 19.3 million cancer cases were newly detected, with an estimated increase of 47% by 2040 (Sung *et al.*, 2021). Proteins inherent to milk have been observed to disrupt cancer cell cycles (Pessione and Cirrincione, 2016). The α - lactalbumin in milk combines with oleic acid to produce a complex with antiproliferative activities (Pessione and Cirrincione, 2016). One study observed that Camel milk exerted cytotoxicity in cancer cells, decreased migration levels, and induced autophagy (Krishnankutty *et al.*, 2018). However, the milk had no significant effect on their apoptosis (Krishnankutty *et al.*, 2018). Other mechanisms of its antiproliferative action have also been studied in depth previously (Habib *et al.*, 2013; Badawy, El-Magd and AlSadrah, 2018; Khan *et al.*, 2021).

Regarding the impact of storage, the antiproliferative effect against HT - 29 was enhanced with an increasing storage period ($p < 0.05$). A similar observation was made on liver cancer cells (Kamal *et al.*, 2018).

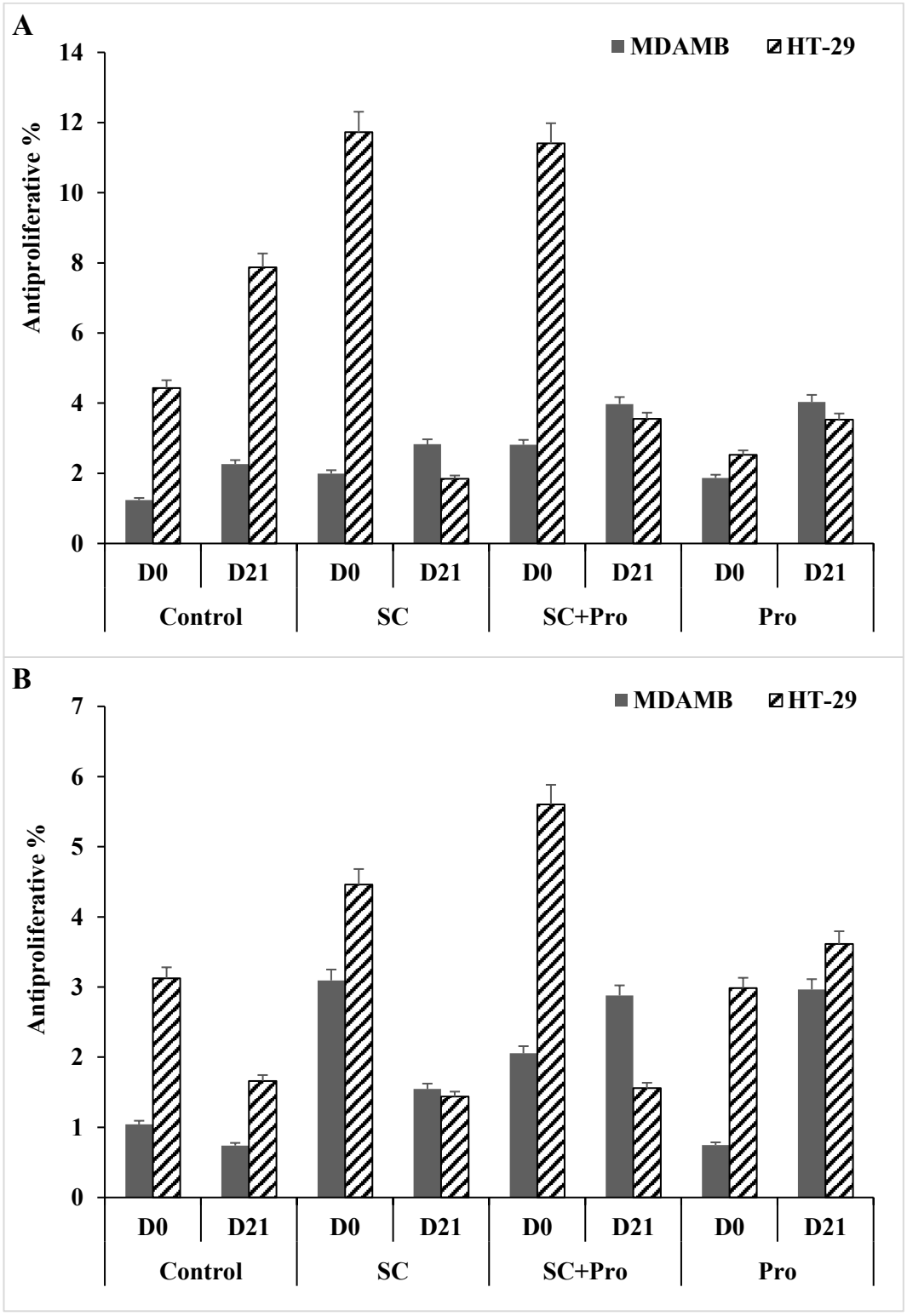

Figure 7**:** Anticancer activity of fermented camel (a) and bovine (b) milk. SC: *Lactobacillus delbrueckii* subsp. *delbrueckii* and *Lactobacillus delbrueckii* subsp. *bulgaricus* Pro: *Lactococcus lactis.* Bars are mean ±SD of n=3

3.8 Variables and Principal Component Analysis of Fermented Camel and Bovine Milk

As shown in Figure 8, a positive correlation between antioxidant activity, α - Amylase and α - Glucosidase inhibition, antiproliferative activity, and total acidity was observed. In contrast, a negative correlation in terms of pH and proteolysis was seen. In Figure 8A, the description of 47.3% and 45.7% of the data is present for fermented camel and bovine milk, respectively. Figure 8 B explains 24.2% and 22.5% of the variations for the two milks, respectively.

In Figure 9, the grouping for the fermented milks was performed based on storage period. According to the PCA biplots, on the first day of storage, both milks fermented with SC, SC + Pro, and Pro were categorized together at a higher position (Blue colored) (Figure 9). In comparison, control samples were towards the lower end. An identical pattern was observed on the last day of storage too. It is evident that milks with similar properties have been grouped together in the plot. The control samples were comparatively lower in the biological activities discussed in the previous sections.

Regarding the type of treatment, SC or SC + Pro treatment resulted in lesser variation than a sole Pro treatment. This implies that adding a Pro did have a significant impact on the final product. Comparing the two milks, Camel milk samples given an SC or an SC + Pro treatment had lesser variance than their Bovine counterparts in the plots (Figure 9). This could be because camel milk did show greater, similar effect as an antioxidant, in OPA analysis, antiproliferative action and in α - Amylase and α - Glucosidase inhibition.

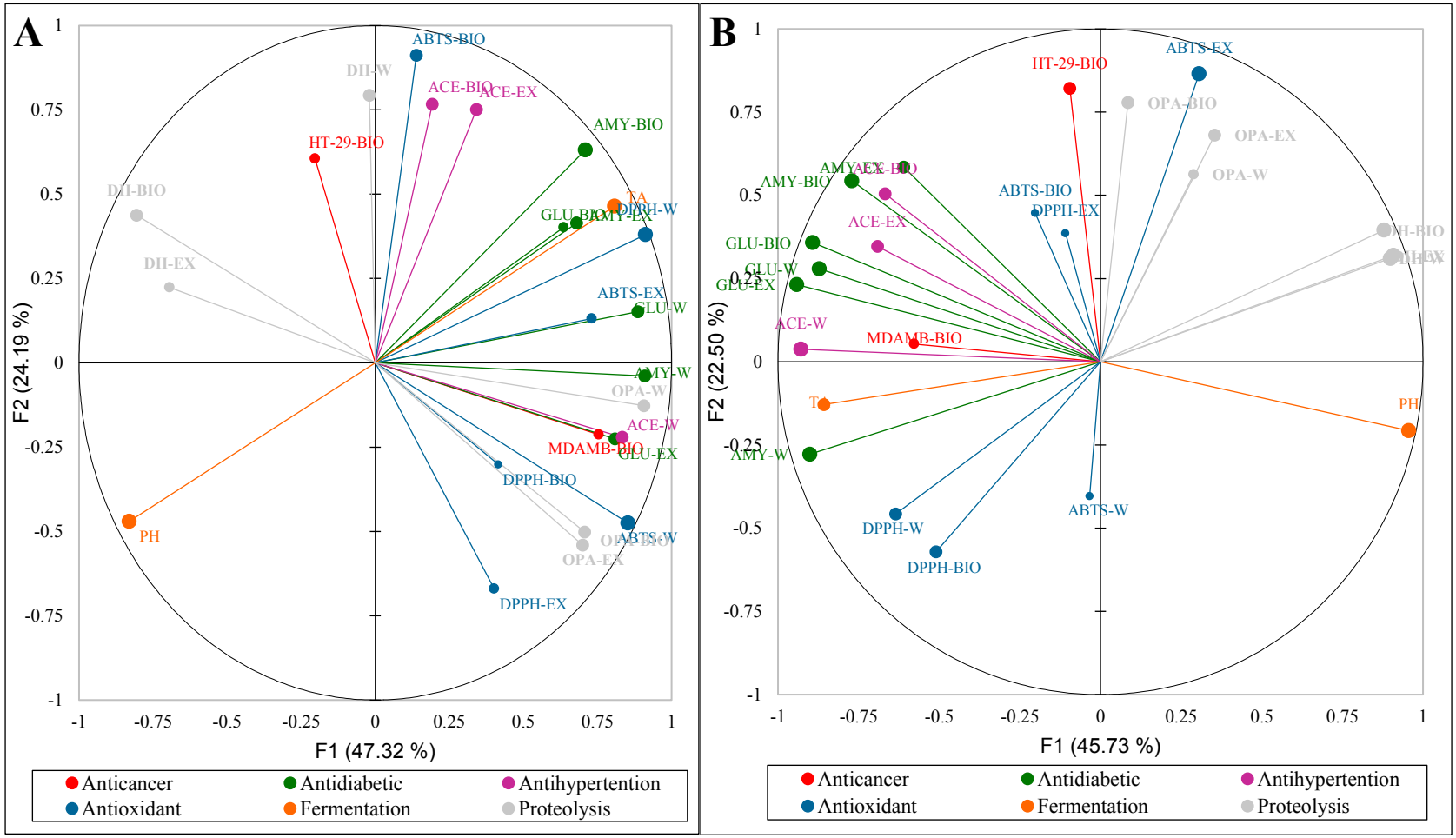

Figure 8: Variables of PCA analysis of fermented camel (a) and bovine milk (b). SC: *Lactobacillus delbrueckii* subsp. *delbrueckii* and *Lactobacillus delbrueckii* subsp. *bulgaricus* Pro: *Lactococcus lactis*. Bars are mean ±SD of n=3

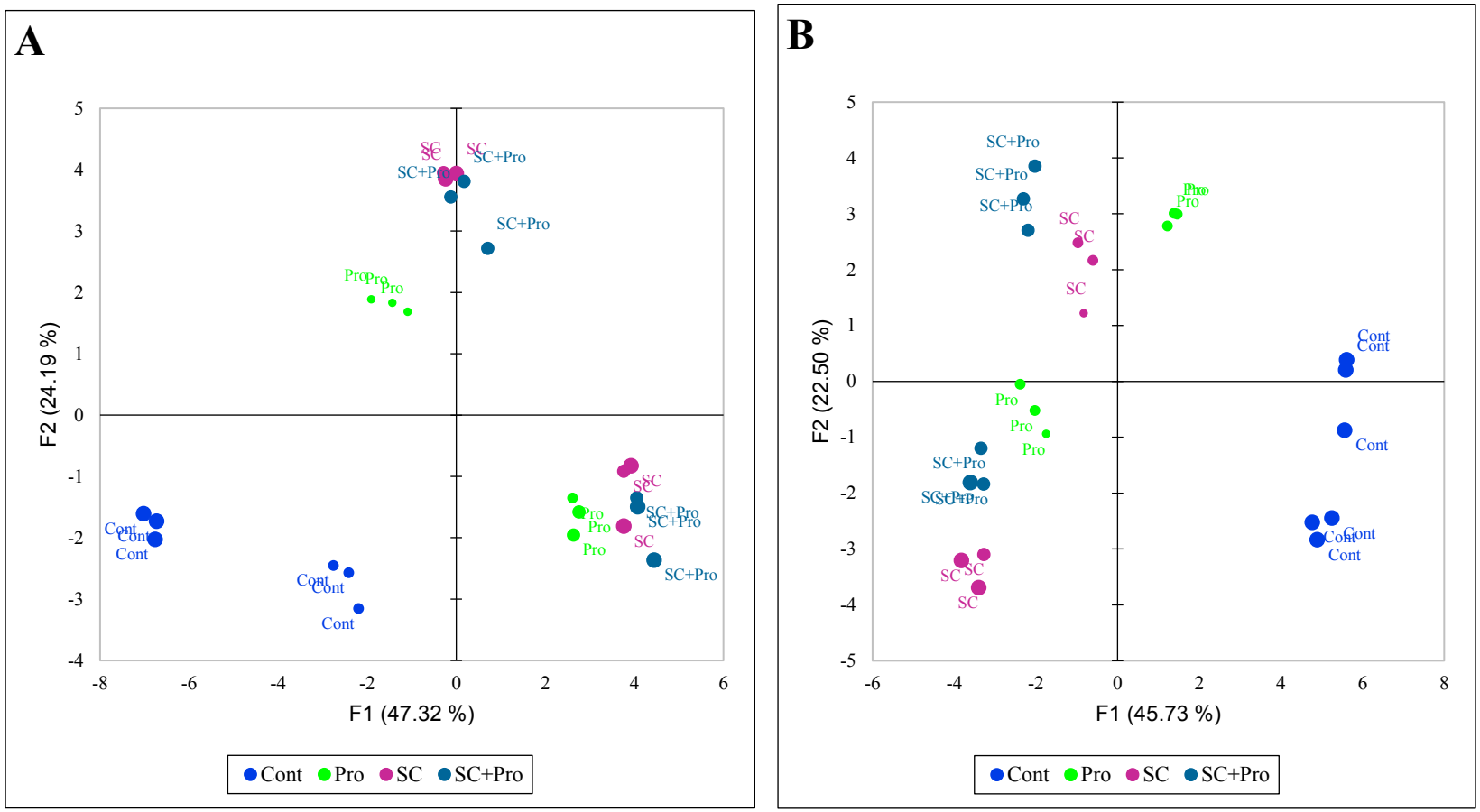

Figure 9: Observations PCA Analysis of Fermented Camel (A) and Bovine Milk (B). SC: *Lactobacillus delbrueckii* subsp. *delbrueckii* and *Lactobacillus delbrueckii* subsp. *bulgaricus* Pro: *Lactococcus lactis*. Bars are mean ±SD of n=3

Chapter 4: Conclusion

This study investigated the biological impact of *in - vitro* digested fermented Camel and Bovine milks, respectively. It was observed that varying the started culture had a limited impact on biological activity. Under *in - vitro* conditions, the WSE of fermented Camel milk had a higher biological effect than fermented Bovine milk. An *in - vitro* digestion, enhanced the biological impact of both the milks and made them susceptible to the proteolytic effect of enzymes. Future research should compare other strains of probiotics and starter cultures for bioactive properties. There lies a possibility that appropriate and efficient fermentation, and an apt mixture of probiotics could result in excellent health benefits. Studies on rat and human models are needed to make firm conclusions.

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List of Publications

Ayyash, M., Abdalla, A., Alhammadi, A., Ranadheera, C.S., Baig, M.A., Al - Ramadi, B., Chen, G., Kamal - Eldin, A. and Huppertz, T., 2021. Probiotic survival, biological functionality and untargeted metabolomics of the bioaccessible compounds in fermented Camel and Bovine milk after *in - vitro* digestion. Food Chemistry, 1 - 10.