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Biochemical characteristics and therapeutic interests of a formulation based on a mixture of dromedary milk-urine

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In front of the jury

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I dedicate this achievement to

My dear father ***Mohammed*** *and my precious mother* ***Yamina*** *who sacrificed their everything so I can stand here today*

My beloved husband ***Imad*** *that supported me during this accomplish*

My little prince, my son ***Ahmed***

My brother ***Nour*** *and sister* ***Rim***

My dear family and my great friends and every person that helped and participated in achieving the present study

SARA

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Sara

Biochemical characteristics and therapeutic interests of a formulation based on a mixture of dromedary milk-urine

Abstract

Culturally, the consumption of dromedary milk and urine and their mixture for being a therapeutic choice against various health disorder is quite popular among the Algerian Saharan population. Moreover, few studies were dedicated on characterizing the dromedary milk (DM) mixed with a small amount of dromedary urine (DU). Therefore, this work aimed to reveal some physical and biochemical characteristics of the DM alone and incorporated with DU at two different concentrations. Furthermore, this work aimed to investigate the neuroprotective, antioxidant, anti-diabetic and antiobesity activities of dromedary milk, urine, and their mixtures by *in vitro* tests. The dromedary milk and urine were collected from healthy females living in the region of Ouargla. The samples were subjected to the physical-chemical and biochemical characterization that consisted on the pH, colour, Brix, fats and minerals evaluating with proteins and sugar content using FTIR; in addition to protein oxidation. All samples were then digested *in vitro* by peptic enzymes. The hydrolysates samples were characterized with HPLC, FTIR, SDS-PAGE and Zetasizer. Moreover, the inhibitory capacity of the digested samples was tested against the activity of AChE, α-amylase, α-glucosidase and pancreatic lipase, in addition to the determination of the antioxidant activity by the ABTS and FRAP assays.

The obtained results showed that DM counts as a rich source of the nutritional element yet its proprieties were affected by the addition of the DU. An important increasing in colour, sugar and protein content with a hight augmentation in the pH, mineral levels in addition to the augmentation in the concentration of the free thiols, where the more urine is added the more milk proteins are oxidated as demonstrated by the characteristics of the two mixtures which can negatively affect DM therapeutical proprieties especially the one related to proteins. Indeed, it was demonstrated by the samples the hydrolysates' characterization that a remarkable molecular interaction was produced after mixing the two liquids. Nevertheless, the recorded results, showed that DM was the most active sample vis-à-vis the selected enzymes with the lowest IC_{50} that was 0.312±0.122, 0.341±0.039mg/ml, 0.478±0.137 and 1.44±0.495 mg/mL for the lipase, AChE, α-glucosidase and α-Amylase, respectively. Followed by the DUH sample that exhibited an IC_{50} of 0.371±0.171mg/ mL and 1.535±0.541 mg/mL against α-glucosidase and α-Amylase, with values of 0.625±0.265 mg/mL and 0.472±0.074mg/ml as IC_{50} for the lipase and AchE, respectively. While the two mixtures hydrolysates demonstrated the lowest ability to block the tested enzymes functionality with the highest IC_{50} in all assays. Nevertheless, DMH was the most active sample hydrolysate compared to the other samples either in radical scavenging activity or in the Fe3+ reducing capacity tests with the miner IC_{50} ($p<0.05$) followed by the DUH, while the two mixture hydrolysates exhibited a very high IC_{50} reflecting a low antioxidant capacity.

Concluding that dromedary milk and urine are a natural source of bioactive substances with a neuroprotective, antidiabetic, antioxidant and antiobesity activities that can be included separately not-mixed in the diet of Alzheimer disease, diabetic patient, people suffering from obesity problems and potentially therapeutic options for health disorders related to the oxidative stress.

Key words: Dromedary milk, dromedary urine, mixtures, hydrolysates, biochemical characterization, therapeutical activities.

Caractéristiques biochimique et intérêts thérapeutiques d'une formulation à base du mélange urine-lait de chamelles

Résumé

Culturellement, la consommation de lait et d'urine de dromadaire et leur mélange comme un choix thérapeutique contre divers troubles de santé est assez populaire parmi la population saharienne algérienne. En plus des faibles recherches sont menées à l'études et la caractérisation du lait de dromadaire (DM) mélangé à des petites quantités d'urine de dromadaire (DU). Par conséquent, l'objectif de notre travail est de révéler certaines caractéristiques physiques et biochimiques du DM seul et incorporé au DU à deux concentrations différentes. Par ailleurs, ce travail vise aussi à étudier les activités neuroprotectrices, antioxydantes, antidiabétiques et anti-obésité du lait de dromadaire, de l'urine et de leurs mélanges par des tests *in vitro*. Le lait et l'urine de dromadaire sont prélevés à partir des chamelles saines vivant dans la région de Ouargla. Les échantillons sont soumis à la caractérisation physico-chimique et biochimique qui consistait à évaluer le pH, la couleur, le Brix, teneur en graisses et en minéraux, ainsi la teneur en protéines et en sucre déterminé à l'aide de FTIR ; en plus de l'oxydation des protéines. Tous les échantillons sont digérés *in vitro* par des enzymes peptiques. Les hydrolysats sont par suite caractérisés par l'HPLC, FTIR, SDS-PAGE et le Zetasizer. De plus, la capacité inhibitrice des échantillons digérés est testée contre l'activité de l'AChE, de l'α-amylase, de l'α-glucosidase et de la lipase pancréatique, en addition de la détermination de l'activité antioxydante par les tests ABTS et FRAP.

Les résultats obtenus montrent que le DM compte comme une riche source des éléments nutritionnels, mais ses propriétés peuvent être affectées par l'ajout de l'urine de dromadaire. En effet, une augmentation remarquable est observée de la couleur, de la teneur en sucre et en protéines avec une forte augmentation du pH, des niveaux de minéraux en plus de l'augmentation de la concentration des thiols libres, où plus on ajoute d'urine, plus les protéines du lait sont oxydées comme illustrée par les caractéristiques des deux mélanges qui peuvent affecter négativement les propriétés thérapeutiques du DM, en particulier celle liée aux protéines. En effet, une interaction moléculaire remarquable est illustrée d'après la caractérisation des hydrolysats des deux formules. Néanmoins, les résultats enregistrés montrent que le DM était l'échantillon le plus actif vis-à-vis les enzymes sélectionnées avec les IC_{50} les plus faibles soit 0.312±0.122mg/ml, 0.341±0.039mg/ml, 0.478±0.137 et 1.44±0.495 mg/ml pour la lipase, l'AChE, l'α-glucosidase et l'α-amylase, respectivement. Suivi par l'échantillon DUH qui a présenté une capacité remarquable avec des IC_{50} de 0.371 ± 0.171 mg/ml et 1.535 ± 0.541 mg/ml contre l'α-glucosidase et l'α-amylase, avec des valeurs de 0.625 ± 0.265 mg/ml et 0.472 ± 0.074 mg/ml comme IC_{50} pour la lipase et l'AChE, respectivement. Alors que les deux mélanges d'hydrolysats ont démontré la capacité la plus faible à bloquer la fonctionnalité des enzymes testées avec l'IC_{50} la plus élevée dans tous les tests. Néanmoins, le DMH était aussi l'échantillon le plus actif par rapport aux autres échantillons dans l'activité antioxydant soit contre le radical d'ABTS soit dans les tests de capacité de réduction de Fe3+ avec l' IC_{50} le plus mineur ($p<0.05$) suivi par le DUH, tandis que les deux hydrolysats de mélange présentaient des IC_{50} très élevée reflétant une faible capacité antioxydante.

Concluant que le lait et l'urine de dromadaire sont une source naturelle de substances bioactives avec des activités neuroprotectrices, antidiabétiques, antioxydantes et anti-obésité qui peuvent être incluses séparément non mélangées dans le régime alimentaire des patients qui souffrent d'Alzheimer, diabétiques, des personnes souffrant de problèmes d'obésité, ainsi comme options potentiellement thérapeutiques pour les troubles de santé liés au stress oxydatif.

Mots clés : Lait de dromadaire, urine de dromadaire, mélanges, hydrolysats, caractérisation biochimique, activités thérapeutiques.

الخصائص البيوكيميائية والقدرات العلاجية لتركيبة تعتمد على مزيج من حليب و بول الإبل

الملخص

يعتبر استهلاك حليب وبول الإبل ومزيجهما خيارًا علاجيًا ضد الاضطرابات الصحية المختلفة خاصة من قبل سكان الصحراء الجزائرية. علاوة على ذلك، تم تخصيص عدد قليل من الدراسات حول توصيف حليب الإبل الممزوج مع البول بكمية صغيرة، لذلك يهدف هذا العمل إلى الكشف عن بعض الخصائص الفيزيائية والكيميائية الحيوية لمركب من مزيج لحليب و بول الإبل بتركيزين مختلفين. إضافة إلى تقصي بعض القدرات العلاجية كالأنشطة الوقائية للأعصاب، القدرة ضد تفاعلات الأكسدة، بالإضافة إلى القابلية العلاجية ضد مرض السكري والسمنة لحليب وبول الإبل ومزيجهما عن طريق الاختبارات المعملية. تم جمع الحليب والبول من ناقات سليمة تعيش في منطقة ورقلة حيث تم إخضاع العينات للتوصيف الفيزيائي والكيميائي والبيوكيميائية، بدءا من الأس الهيدروجيني، الخصائص اللونية، البريكس والدهون، المعادن، مع البروتينات ومحتوى السكر باستخدام تقنية FTIR بالإضافة إلى أكسدة البروتين. بعد ذلك تم هضم جميع العينات في المختبر بواسطة إنزيمات هضمية، ثم تم دراسة خصائصا باستخدام HPLC وFTIR وSDS-PAGE وZetasizer. علاوة على ذلك، تم اختبار السعة التثبيطية للعينات المهضومة ضد نشاط بعض الإنزيمات المتمثلة في الأسيتيل كولين إستراز، الألفا-أميلاز، الألفا-جليكوسيداز و الليباز البنكرياسي بالإضافة إلى تحديد نشاط مضادات الأكسدة عن طريق مقياسات FRAP و ABTS.

أظهرت النتائج المتحصل عليها أن حليب الإبل مصدر غني بالعناصر الغذائية ولكن خصائصه تأثرت بإضافة كمية من بول الإبل له، حيث طرأت عليه تحولات مهمة في محتوى اللون والسكر والبروتين مع زيادة عالية في درجة الحموضة، ومستويات المعادن بالإضافة إلى زيادة تركيز الثيول الحر، حيث كل ما يتم إضافة المزيد من البول ، يتأكسد المزيد من بروتينات الحليب كما يتضح من خصائص المزيجين و هذا قد يؤثروا سلبًا على الخصائص العلاجية خاصة تلك المتعلقة ببروتينات الحليب. في الواقع، من خلال تشخيص العينات المهضومة تم إثبات أنه تم ظهور تفاعل جزيئي ملحوظ بين مكونات السائلين. كما أظهرت النتائج المسجلة أن ناتج هضم الحليب هو العينة الأكثر نشاطًا ضد الإنزيمات المختارة مع أدنى تركيزات تثبيطية و التي كانت 0.312± 0.122 مجم / مل ضد الليباز البنكرياسي، 0.039± 0.341مجم / مل ضد الأسيتيل كولين، 0.0137±0.478 مجم / مل ضد الجليكوزيداز و 1.44±0.495 مجم / مل ضد الأميلاز، متبوعًا بعينة البول التي أظهرت IC50 0.171 ± 0.371 مجم / مل و 1.535 ± 0.541 مجم / مل مقابل الجليكوزيداز و الأميلاز، إضافة إلى 0.625 ± 0.265 مجم / مل و 0.472 ± 0.074 مجم / مل كIC50 ضد لليباز و الأسيتيل كولين إستراز على التوالي. بينما أظهرت نتائج الخليطين أقل قدرة على منع وظيفة الإنزيمات المختبرة مع أعلى نسبة IC50 في جميع المقياسات. ليس هذا فقط ، لقد أظهرت العينات المهضومة للحليب أنها الأكثر نشاطًا مقارنة بالعينات الأخرى في المقياسات المختارة في تفاعلات الأكسدة متبوعًا بعينات البول، بينما أظهر المزيجين قدرة منخفضة من مضادات الأكسدة.

يستخلص من الدراسة المنجزة إلى أن حليب وبول الإبل مصدر طبيعي للمواد النشطة بيولوجيًا مع أنشطة وقائية للأعصاب ومضادة لمرض السكر ومضادات الأكسدة ومضادة للسمنة والتي يمكن تضمينها بشكل منفصل وليس ممزوجًا في النظام الغذائي لمرض الزهايمر ومرضى السكري والأشخاص الذين يعانون من مشاكل السمنة والخيارات العلاجية المحتملة للاضطرابات الصحية المتعلقة بالإجهاد التأكسدي.

الكلمات المفتاحية: حليب الإبل، بول الإبل، المزيج، التوصيف الكيميائي الحيوي، العينات المهضومة، الأنشطة العلاجية.

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ABREVIATIONS LIST

ABTS	**:**	2,2’-azinobis-(3-ethylbenzothiazoline-6-sulfonate
Ach	**:**	Acetylcholine
AChE	**:**	Acetylcholinesterase
AD	**:**	Alzheimer's Disease
ANOVA	**:**	Analysis of Variance
BMI	**:**	Body Mass Index
CNS	**:**	Central Nervous System
CSA	**:**	Camel Serum Albumin
DM	**:**	Dromedary Milk
DMH	**:**	Dromedary milk hydrolysate
DNS	**:**	3,5-dinitro salicylic acid
DNTB	**:**	5,5'-dithiobis-(2-nitrobenzoic acid)
DU	**:**	Dromedary Urine
DUH	**:**	Dromedary urine hydrolysate
FRAP	**:**	Ferrique Reducing Antioxydant Power
FTIR	**:**	Fourier Transformation InfraRed spectroscopy
HPLC	**:**	High Performance Liquid Chromatography
IC50	**:**	Inhibitory concentration at 50%
ICP-MS	**:**	Inductively Coupled Plasma Mass Spectrometry
Ig	**:**	Immunoglobulins
IR	**:**	Inhibition Ratio
PCA	**:**	Principal Component Analysis
pH	**:**	Potential of hydrogen
pNPG	**:**	p-nitrophenyl-α-d-glucopyranoside
PNP	**:**	P-nitrophenyl
PP3	**:**	Proteose Peptone 3
SDS-PAGE	**:**	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SH	**:**	Sulfhydryl Groups
SPSS	**:**	Statistical Package for the Social Sciences
T2D	**:**	Type 2 diabetes
TNB	**:**	5-thio-2-nitrobenzoate
EDTA	**:**	Ethylene diamine tetra-acetic acid
TSS:	**:**	Total soluble solids

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Introduction

Introduction

Le *Camelus dromadaires* connu aussi par « chameau à une bosse » est l'un des animaux les plus appréciés par les peuples arabes et les bédouins en général et les populations sahariennes algériennes en particulier. À l'échelle mondiale, le lait de dromadaire et ses produits laitiers ainsi sa viande sont largement consommés non seulement en Afrique et dans les États arabes, mais aussi très appréciés en Asie orientale et centrale, en Amérique latine et même en Australie **(Alhadrami and Faye, 2022, Oselu et al., 2022)**. Son adaptation aux conditions climatiques difficiles de ses zones d'abondance est la cause et l'origine de son utilité et de son utilité **(Ho *et al.*, 2021)**.

En effet, grâce à ses propriétés physiologiques externes et internes, en plus de la fonctionnalité unique de ses organismes et de ses voies métaboliques, le dromadaire est capable de maintenir ses besoins et demandes biologiques malgré les sources de nourriture et les quantités d'eau très limitées dans ses régions de localisation, notamment en période chaude **(Medjour, 2014)**.

Le dromadaire est connu par de multiples propriétés qui lui confèrent la capacité de s'adapter aux différentes conditions des zones sahariennes et arides (Figure. 1).

Au cours des dernières décennies, l'importance des productions des dromadaires s'est largement fait remarquer dans la littérature, précisément le lait de dromadaire (DM) **(Dubey *et al.*, 2015)**. Un produit qui est devenu l'un des sujets les plus étudiés dans le domaine des aliments fonctionnels en raison de sa valeur nutritionnelle précieuse et a ces composants qui offrent des fonctions biologiques très importantes à l'humanité **(Górska-Warsewicz *et al.*, 2019 ; Keyvan, 2021 ; McCormick, 2003)**.

En effet, le dromadaire joue un rôle majeur pour fournir aux habitants du désert un lait de bonne qualité nutritionnelle dans des conditions extrêmement difficile, une haute température, de sécheresse et de manque de pâturage **(Medjour, 2014)**. C'est pour cette raison, le lait de dromadaire avait occupé une large place dans les recherches de la section laitière à plusieurs niveaux en raison de ses propriétés physiques, de sa composition chimique, de ses caractéristiques fonctionnelles et de son utilité thérapeutique **(Mostafa *et al.*, 2018 ; Rahmeh *et al.*, 2019 ; Swelum *et al.*, 2021 ; Minich *et al.*, 2022)**.

Malheureusement, peut d'information précise sur la production exacte de ce lait est disponible. En effet, en milieu désertique, il est difficile, parfois impossible, de collecter des données fiables et précise sur la production de DM **(Cardellino *et al.*, 2004 ; Ho *et al.*, 2021)**. Mentionnant que la production de ce liquide est associée de manière directe à multiples facteurs

tel que la durée de lactation, l'alimentation et les conditions du troupeau jouant un rôle important dans la collection des données **(Cardellino *et al.*, 2004)**.

En plus de ces facteurs, le type d'élevage joue aussi un rôle clé dans le recueil et la fiabilité des informations de production du DM. En effet, les dromadaires sont soumis à deux systèmes d'élevage, le type extensif qui est basé exclusivement sur la végétation naturelle et l'élevage intensif qui est basé sur l'utilisation de compléments alimentaires, dans lequel la production laitière est estimée d'être deux fois plus élevée que dans le système d'alimentation extensif **(Field, 1979 ; Medjour, 2014)**.

Par allier, selon plusieurs auteurs, l'amélioration des conditions alimentaires peuvent prolonger la période de lactation et augmenter la quantité de lait produit par cet animal **(Bekele *et al.*, 2011 ; Musaad *et al.*, 2013 ; Oselu *et al.*, 2022)**.

Néanmoins, la disponibilité alimentaire est liée principalement à la variabilité saisonnière (facteurs climatiques : chaleur, aridité) affecte les performances laitières de cet animal **(Medjour, 2014)**. En effet, une variation remarquable est notée dans les différentes saisons où la production laitière atteindre le taux le plus bas en fin de saison sèche **(Faye, 2004)**.

Malgré le manque des données exacte sur la production litière par le dromadaire, le DM est considéré comme l'un des aliments riches les plus importants dans les zones désertiques en raison de sa composition spécifique et équilibrée dans tous les nutriments **(Hammam *et* Agriculture, 2019)**. En plus, les propriétés physiologiques, biologiques et les voies métaboliques du *Camelus dromedarius* influencent la biosynthèse de ce fluide biologique en produisant un liquide unique et hautement homogénéisé riche en molécules bioactives **(Ali *et al.*, 2019)**.

D'une façon générale, le DM se caractérise par une couleur blanche très opaque, due à sa faible composition en graisses et en caroténoïdes **(Yagil et Etzion, 1980 ; Farah et al, 1992 ; Kappelle, 1998)**. Un goût légèrement sucré, acide et même salé avec une saveur un peu sucrée par rapport aux autres laits en raison de son pH qui varies généralement de 6.2 à 6.8 **(Farah, 1993 ; Farah, 2004 ; Al Haj *et* Al Kanhal, 2010 ; Mal *et* Pathak, 2010 ; El Imam Abdalla, 2012 ; Parajapati *et al.*, 2012)**.

De plus, ce lait est moins dense par rapport au lait de vache. Son point de congélation varie de 0.57°C à -0.61°C, avec une viscosité également inférieure à celle du lait de vache **(Kamoun, 1995 ; Kherouatou *et al.*, 2003 ; Al Haj *et* Al Kanhal, 2010 ; Ghennam *et al.*, 2007 ; Faye *et al.*, 2008)**.

La composition chimique du lait de dromadaire comprend les cendres et les solides totaux variant entre 0.7 à 0.95 % et 12.1 à 15 %, respectivement **(Konuspayeva, Faye, & Loiseau, 2009)**. La teneur en protéines varie généralement entre 3.5 à 4.5%, les lipides sont constitués de 3.07 à 5.50% et le lactose entre 3.4 à 5.6% **(Sawaya *et al.*, 1984 ; Zhang *et al.*, 2005 ; Zibaee *et al.*, 2015 ; Jilo *et* Tegegne, 2016 ; Pak *et al.*, 2019 ; Rahmeh *et al.*, 2019)**. De plus, le lactose de ce lait diffère du lactose du lait des autres mammifères par sa facilité à être métabolisé par les personnes présentant des cas d'intolérance au lactose **(Hanna J *et al.*, 2001)**.

La partie lipidique du DM n'est pas trop recherchée et étudiée tel que les autre constituant, où peu de travaux ont été menés sur l'étude des propriétés physico-chimiques et structurales de la matière lipidique de ce lait **(Hadddin, 2008 ; Parajapati *et al.*, 2012**). Cependant, La partie lipidique du DM a montré une forte corrélation positive avec sa teneur en protéines **(Parajapati et al., 2012)**.

Les peux travaux menés sur la caractérisation des lipides de ce lait rapportent que les triglycérides représentent 96% des lipides totaux. Par ailleurs, parmi le complexe lipidique, les phospholipides de ce lait sont constitués d'acides gras contenant majoritairement des acides gras avec des insaturations et correspondant à de longues chaînes d'atomes de carbone, représentés principalement par les acides palmitique et stéarique. Alors que les acides gras à chaînes courtes sont relativement rares **(Ho *et al.*, 2021 ; Gorban *et* Izzeldin, 2001 ; Mohamed *et* Mustafa, 2013 ; Tasturganova *et al.*, 2018)**. Néanmoins, la variation lipidique du DM entre les acides gras saturés et insaturés participe à l'amélioration de la qualité générale du lait et à sa bonne homogénéisation avec les protéines **(Yagil *et al.*, 2000 ; Mohamed *et* Mustafa, 2013 ; Tasturganova *et al.*, 2018).**

À l'échelle mondiale, les protéines de lait de dromadaire sont devenues jusqu'à présent le contenu principal des chercheurs. De manière réaliste, en raison de leur apport nutritionnel (source d'acides aminés essentiels) et de leurs propriétés techno-fonctionnelles particulières, dans lesquelles ils sont considérés comme plus spéciaux par rapport aux autres protéines du lait, les protéines de lait de dromadaire sont devenues la partie la plus recherchée et étudiée chercheurs jusqu'à présent **(Parajapati *et al.*, 2012)**.

En fonction de leur solubilité en milieu acide, les protéines de DM se répartissent comme pour les autres laits, en deux fractions : les caséines et les protéines de lactosérum (Al Haj et Al Kanhal, 2010). Les caséines précipitent les caséines de dromadaire sont comptées

majoritairement comme des phosphoprotéines qui représentent la fraction protéique la plus abondante du DM, avec une abondance de 73 à 81 % des protéines totaux **(Al Haj *et* Al Kanhal, 2010)**. Ces protéines sont divisées en trois groupes : les α-caséines (α-S1 et α-S2), les β-caséines et les κ-caséines qui se sont avérées deux fois plus faibles par rapport à celle des caséines bovins **(Al Haj *et* Al Kanhal, 2010 ; Mal *et* Pathak, 2010 ; El Imam Abdalla, 2012)**.

D'autre part, les protéines de lactosérum du DM représentent 18.5 à 27% des protéines totales, à l'exception de la β-lactoglobuline qui a été signalé d'être absent dans ce lait par rapport aux autres laits. Pour cette raison, ce lait est compté avec peu ou pas d'effets allergiques **(Sood *et* Sidhu, 1979 ; Mehaia *et al.*, 1995 ; Merin *et al.*, 2001 ; Al-Alawi *et al.*, 2011 ; El-Agamy *et al.*, 2009)**. Cependant, certains travaux ont mentionné la présence de β-lactoglobuline en teneur très minoritaire dans certains cas **(El-Agamy *et al.*, 2009)**.

Les protéines de lactosérum du DM sont constituées d'α-Lactalbumine, d'albumine sérique de chameau (CSA), d'immunoglobulines (Ig), de protéose-peptones, de lactoferrine, de lacto-peroxydase et de lysozyme **(El agamy *et al.*, 1996 ; Ochirkhuyag *et al.*, 1998 ; Kappeler *et al.*, 1999 et b ; EL-agamy, 2000 ; Girard *et* al, 2000)**. Les trois immunoglobulines identifiées dans ce lait sont les IgG, IgM et IgA avec une prédominance de classe G, un anticorps de masse très élevée, inhibant son transfert via le placenta, par conséquence il est offert aux nouveau-nés par la consommation de lait, précisément le colostrum **(EL-agamy *et al.*, 1996 ; EL-agamy, 2000 ; Kinne *et al.*, 2001 ; El-Hatmi *et al.*, 2006)**.

En fait, il est rapporté par multiples recherches que la concentration d'IgG atteint son maximum au cours de la première période de lactation (7 premiers jours de lactation) pour protéger le nouveau-né de différentes infections **(Kinne *et al.*, 2001 ; El-Hatmi *et al.*, 2006 ; Konuspayeva *et al.*, 2007 ; Makkawi *et al.*, 2019 ; El-Hatmi *et* Levieux, 2006 ; Al-Youcef *et al.*, 2012)**. Par conséquence, une grande partie de l'activité antimicrobienne du DM est liée à son contenu important en immunoglobulines G, en plus de la présence de lactoferrine et de lysozyme **(Ikeda *et al.*, 2000 ; Kinne *et al.*, 2001 ; El-Hatmi *et al.*, 2006 ; Benkerroum *et al.*, 2004 ; Redwan *et al.*, 2014)**. En effet, il a été rapporté que les IgG de ce lait représentent une activité très antivirale, notamment contre les rotavirus **(El-agamy *et al.*, 2012)**.

Par ailleurs, la protéose peptone 3 (PP3) est caractérisée par une capacité à inhiber la lipolyse spontanée du lait et à stimuler la mitogénicité des cellules d'hybridomes et la croissance des souches du bifidus-bactériennes **(Mati *et al.*, 1993 ; Girardet *et al.*, 2000b ; Mahmoudi *et al.*, 2016)**.

En plus de la fonctionnalité importante des protéines du DM dans leur forme principale, les peptides libérés à partir de ces protéines, que ce soit avec la digestion *in vivo* ou *in vitro*, sont considérés comme un choix thérapeutique potentiel pour plusieurs problèmes de santé **(Ebaid *et al.*, 2015)**.

En effet, jusqu'à présent de multiples activités sont fondées à être assurées par les peptides du DM après leur hydrolyse avec la pepsine, la trypsine et la chymotrypsine telles que des activités antioxydantes, antihypertensives, anti-inflammatoires et immunomodulatrices **(Ebaid, *et al*,. 2014 ; Ebaid *et* Ahmed, 2013)**. Ainsi que les capacités anti-infectieuses et anticytotoxiques que ces peptides peuvent garantir au corps humain (**Salwa *et* Lina, 2010 ; Almahdy *et al* ., 2011 ; El-Fakharany *et al* ., 2012 ; Mal *et* Sahani, 2006 ; Mona *et* Mosa, 2010)**.

Néanmoins, la teneur en vitamines du DM fait encore l'objet de recherches. Jusqu'à présent, il est documenté que ce lait contient moins de vitamine A, de riboflavine, de thiamine, d'acide folique et d'acide pantothénique que le lait de vache **(Algaithi *et al.*, 2022 ; Farah *et al.*, 1992; Faye *et al.*, 2019 ; Tastemirova *et al.*, 2022)**. D'autre part, la concentration de pyridoxine, de vitamine B12 et de vitamine E se retrouvent au même niveau entre les laits de dromadaire et de vache. Cependant, la vitamine C, qui est un puissant antioxydant, est enregistré comme étant plus élevée dans le DM en comparant aux autres laits **(El-Fakharany *et al.*, 2012 ; Mahmoud *et* Ahmed, 2013 ; Ebaid *et al.*, 2015 ; Zhao *et al.*, 2015)**.

La partie minérale du DM n'est pas bien étudiée. Seuls quelques éléments inorganiques sont étudiés et leur quantité exacte est documenté. Les minéraux les plus recherchés dans le lait de dromadaire est le calcium, le sodium, le potassium, le magnésium, le chlorure, le phosphore et le zinc, qui se trouvent à une concentration notable **(Shamsia, 2009)**.

À l'échelle mondiale, en raison de ses propriétés anticancéreuses et antidiabétiques, de sa teneur élevée en acides gras insaturés et de son faible effet hypoallergénique, le lait de dromadaire frais et fermenté est désigné comme une importante source de guérison naturelle pouvant être utilisée à des objectives médicinales pour plusieurs états pathologiques **(Konuspayeva *et al.*, 2009 ; Al-Juboori *et al.*, 2013 ; Zhao *et al.*, 2015)**.

Le lait n'est pas le seul produit biologique que le dromadaire produit avec cette originalité. L'urine de dromadaire (DU) est également utilisée depuis longtemps comme ingrédient de guérison traditionnel par la population saharienne algérienne. Le recyclage de l'urée et de l'ammoniac par les reins de cet animal à cause des condition vigoureux rend cette urine moins ou non toxique **(Schmidt-Nielsen, 1979 ; Read, 1995)**.

Malgré l'utilisation populaire de l'DU dans les cultures arabes et musulmanes, un manque important de données est enregistré sur ce produit. En effet, peu d'informations sont documenté sur la composition exacte et les propriétés de l'urine de dromadaire. Il est constaté que ces urines se caractérisent par leur richesse en potassium, phosphore et magnésium, trois ions connus pour leurs effets thérapeutiques ainsi que la présence des traces des protéines, de triglycérides et de cholestérol qui sont généralement absents des urines humaines et animales **(Hasni *et al.*, 2015 ; Hasni *et al.*, 2022)**.

Il est également documenté que l'urée, principal composant de l'urine connu pour sa toxicité, n'est présente qu'à l'état de traces dans les urines de chameau. Des composés phénoliques tels que les polyphénols, mais les flavonoïdes ont été détectés en faible quantité notable **(Hasni *et al.*, 2022)**.

Jusqu'à présent, il est rapporté que ce liquide biologique possède des propriétés antibactériennes, antifongiques et antivirales en raison de certaines de ses compositions chimiques **(Al-Yousef *et al.*, 2012)**. De plus, **Agarwal *et al.*, (2009)** ont rapporté qu'il peut être utilisé pour traiter la neuropathie diabétique.

Néanmoins, l'DU également montre une capacité antiplaquettaire, une capacité inhibitrice contre les cellules tumorales et cancéreuses *in vitro*, ainsi la force de bloquer l'induction de Cyp1a1 qui est un gène activateur du cancer **(Alhaidar *et al.*, 2011 ; Syed *et al.*, 2015 ; Alhaider *et al.*, 2017)**. Par ailleurs, **Khorshid *et* Moshref (2006)** ont rapporté l'effet anticancérigène de cette urine dans différents types de cancer chez le rat.

L'évaluation de l'activité antioxydante de l'DU par les tests ABTS, DPPH, FRAP et PPM a montré que ce liquide exprime une activité antioxydante très importante **(Hasni et al., 2015 ; Hasni et al., 2022)**. Néanmoins, il est constaté que cette urine possède une très faible activité hémolytique confirmant sa non-toxicité pour le corps humain en cas de consommation **(Hasni *et al.*, 2015)**.

L'urine de dromadaire est considérée comme un produit « miraculeux » utilisé en médecine prophétique, depuis l'ère préislamique où le lait et l'urine de chamelle sont utilisés comme médicament alternative à boire pour différents problèmes de santé **(Konuspayeva *et al.*, 2009, Omar *et al.*, 2010 , Andreja *et al.*, 2015)**.

Malgré la difficile acceptation de la consommation d'urine, mais pour la population bédouine des régions arides et semi-arides, le lait de dromadaire n'est pas toujours consommé seul, mais proportionnellement, mélangé à une petite quantité d'urine de dromadaire, qui est considérée comme une solution efficace et pour multiples troubles de santé **(Kinne *et al.*, 2001 ; El-Hatmi *et al.*, 2006 ; Konuspayeva *et al.*, 2007 ; Al-Youcef *et al.*, 2012)**.

Les deux produits DM et DU sont classés comme ingrédient très important dans la médecine naturelle ou la pharmacologie traditionnelle, qui est l'option la plus utilisée pour de nombreuses personnes et dans plusieurs cultures pour régler les troubles de santé internes et externes (**Al Haj et Al Kanhal, 2010 ; Mal et Pathak, 2010 ; El Imam Abdalla, 2012)**.

Une grande partie de la médecine naturelle est basée sur le contrôle des activités enzymatiques liées aux troubles de santé internes comme les problèmes qui deviennent trop courants dans le monde entier, comme le diabète et l'obésité, en activant ou en inhibant la fonctionnalité enzymatique responsable de ces maladies **(Manna *et* Jain , 2015 ; Barr, 2018 ; Galice-Garcia *et al.*, 2020)**.

Le diabète, une des maladies les plus répandues dans le monde qui est un trouble de l'assimilation, de l'utilisation et du stockage des sucres apportés par les aliments qui survient lorsque le pancréas ne produit pas assez d'insuline (diabète de type I) ou lorsque l'organisme est incapable d'utiliser l'insuline, il produit efficacement, entraînant une accumulation de glucose dans le sang connu sous le nom de diabète de type 2 (T2D) qui est la forme la plus courante de la maladie, qui au fil du temps entraîne de graves dommages à de nombreuses parties du corps, en particulier les nerfs et le sang navires **(Fong *et al.*, 2004 ; Gudmundsson *et al.*, 2007 ; Chobot *et al.*, 2018 ; Quintero *et al.*, 2018 ; Saeedi *et al.*, 2019)**.

Cependant, les raisons de l'apparition de DT2 sont encore inconnues. Il existe plusieurs facteurs de risque importants, tel que l'obésité qui est définie comme une accumulation très importante de graisse dans le corps affectant la santé générale **(Mobbs, 2014 ; Chooi *et al.*, 2019 ; Blüher, 2020)**. L'obésité est souvent estimée par l'indice de masse corporelle (IMC), **(Mobbs, 2014 ; Peltz *et al.*, 2010 ; Zhao *et al.*, 2018)**. Dans laquelle, plus l'IMC augmente, plus les risques de développer un DT2 augmentent en retour. Pour ces raisons, la prévention de la prise ou de la perte de poids chez les personnes atteintes de DT2 devrait être la principale cible thérapeutique **(Wilding, 2014 ; Franz, 2017 ; Apovian *et al.*, 2019)**. Le diabète et l'obésité peuvent être gérés en contrôlant l'activité de certaines enzymes impliquées dans la digestion et le métabolisme du sucre et des graisses, telles que l'α-amylase, l'α-glucosidase et la lipase **(Tucci *et al.*, 2010 ; Zielińska *et al.*, 2020 ; Oluwagunwa *et al.*, 2021 ; Uddin *et al.*, 2022)**.

Les enzymes α-amylase et α-glucosidase sont responsables de la production des monosaccharides libres, en particulier le glucose qui se déplace des intestins vers la circulation sanguine. L'α-amylase agit sur l'hydrolyse des polysaccharides tels que l'amidon en di et oligosaccharides qui sont soumis à l'hydrolyse en monosaccharides par l'α-glucosidase **(Kinne *et al.*, 2001 ; El-Hatmi *et al.*, 2006 ; Konuspayeva *et al.*, 2007 ; Makkawi *et al.*, 2019 ; gs ;**

De Wit, 1998 ; El-Hatmi *et* Levieux, 2006 ; Al-Youcef *et al.*, 2012). La fonctionnalité d'une ou des deux enzymes aide à diminuer la concentration des monosaccharides comme le glucose dans le système interne du corps, contrôlé par sa l'hyperglycémie et le DT2 **(Kinne *et al.*, 2001 ; El-Hatmi et al., 2006 ; De Wit, 1998 ; El-Hatmi *et* Levieux 2006 ; Al-Youcef *et al.*, 2012).**

Par ailleurs, la lipase l'enzymes responsable de l'hydrolyses des graisses alimentaires est également classée comme une enzyme importante dans la gestion de l'obésité qui augmente le risque de développer un DT2. La résistance à l'action de l'insuline (résistance à l'insuline) est fréquemment associée à un excès de graisse corporelle et constitue un défaut précoce du développement du DT2. Par conséquence, l'inhibition de lipase minimise la teneur en lipides absorbé par le corps et stocké au niveau des tissu adipeux **(Kinne *et al.*, 2001 ; El-Hatmi *et al.*, 2006 ; Konuspayeva *et al.*, 2007 ; Makkawi *et al.*, 2019 ; De Wit, 1998 ; Al-Youcef *et al.*, 2012)**.

En outre, la médecine naturelle est aussi orientée vers les maladies neuro-dommageables telles que la maladie d'Alzheimer, connue comme la maladie neurodégénérative caractérisée par des déficits de mémoire progressifs et une altération de la fonction cognitive **(Moya-Alvarado *et al.*, 2016)**.

La maladie de neurodégénérescence Alzheimer est causée à la fois par l'hyperactivité de l'acétylcholine estérase (AChE) qui hydrolyse l'ACh avec un renouvellement de 1 000 à 20 000 molécules par seconde (selon les espèces) et par la détérioration des cellules neurales provoquée par l'activité des radicaux libres dans le système neuronal **(de Oliveira et al., 2012)**.

En plus de la maladie d'Alzheimer, diverses pathologies et troubles de la santé tels que la maladie de Parkinson, les maladies cardiovasculaires, les troubles immunologiques, la polyarthrite rhumatoïde, le diabète ou le développement de cancers et de cellules tumorales, ainsi que des maladies rénales pourraient être produites par une source de stress oxydatif et l'activité des radicaux libre et espèces réactives de l'oxygène **(Maddu, 2019).**

En fait, les substances antioxydantes font partie des objectifs de recherche scientifiques dans plusieurs domaines tels que l'alimentation et la nutrition, la médecine traditionnelle, la phytochimie, la pharmacologie afin de prévenir les dommages causés par les espèces réactives de l'oxygène (ROS) qui pourraient être atteint par un mécanisme systématique contrôlant la capacité redox ou piégeant les radicaux libres ou les ions réducteurs.

Jusqu'à récemment, plusieurs études sont consacrées à l'étude du lait de dromadaire et de ses propriétés générales à plusieurs niveaux, alors qu'aucune étude n'a été consacrée à révéler les éventuels changements pouvant influencer les propriétés physiques et biochimiques du lait

après avoir combiné avec l'urine de dromadaire malgré sa large consommation et utilisation comme produit de guérison par les Bédouins africains, en particulier les Algériens.

À notre connaissance, il n'y a pas d'études menées sur l'importance d'inhiber certaines enzymes comme l'AChE en utilisant du lait de dromadaire qui est utilisé chez les cultures des bédouins comme un ingrédient pharmaceutique alternative, c'est pourquoi cette étude est réalisée. Par ailleurs, jusqu'à présent, plusieurs types de recherches sont consacrées à l'effet antidiabétique et anti-obésité du DM, mais dans la littérature aucune étude est menée sur l'investigation de la capacité du DM tel que consommé principalement par les tribus sahariennes, mélangé à l'DU contre les enzymes liées au diabète, à l'obésité et à la MA.

Dès lors, et compte tenu des points mis en évidence un problème important s'est posé, si le lait et l'urine de dromadaire sont combinés, quelles seraient les caractéristiques biochimiques de cette formulation ? De plus, cette formulation aura-t-elle un intérêt thérapeutique similaire aux intérêts des deux liquides séparés, ou y aura-t-il un développement de nouvelles activités biologiques ?

Le but de la thèse

Les objectifs de cette recherche sont d'abord, d'étudier séparément les caractéristiques biochimiques du lait et de l'urine de dromadaire et de réaliser une formulation à base d'un mélange de ces deux fluides biologiques pour trouver s'il y aura une association et une combinaison des constituants du lait avec les constituants du l'urine recherchant la complémentarité possible entre leurs composants organiques. De plus, pour étudier certaines forces thérapeutiques que les deux liquides peuvent offrir indépendamment et combinées comme les activités neuroprotectrices, antidiabétiques, anti-obésité et antioxydantes.

Structure de la thèse

Pour atteindre les objectifs de la présente étude et pour couvrir le point mis en évidence, ce manuscrit est organisé en trois grandes sections :

• La première est consacrée à une introduction incluant des généralités sur le lait et l'urine de dromadaire, leurs propriétés et bénéfices généraux pour la santé humaine.

• La seconde partie constituée de la partie méthodologique illustre une partie synthétique sur les matériels utilisés et adoptés ainsi qu'une description générale des protocoles suivis

• La troisième section porte sur l'ensemble des résultats obtenus, suivi de discussions. La thèse est terminée par une conclusion globale ainsi que quelques perspectives pour sa continuité.

Nouveautés de la recherche

Le présent travail a abouti à une nouvelle découverte, dans laquelle les objectifs n'ont jamais été rapportés qui sont les suivants :

- La caractérisation des hydrolysats de lait de dromadaire par des enzymes peptiques (in vitro) comme par le FTIR et le Zetasizer.
- La caractérisation d'urine de dromadaire avant et après digestion in vitro avec FTIR, Zetasizer, HPLC.
- Le dosage et l'identification de certains composés phénoliques dans les hydrolysats de lait de dromadaire et d'urine.
- Cette recherche met en évidence une des capacités indéterminées des hydrolysats de lait de chamelle qui est la capacité neuroprotectrice en inhibant et en diminuant l'activité de l'enzyme acétylcholine-estérase (AChE) responsable de la maladie d'Alzheimer.
- La détermination des activités neuroprotectrices, antidiabétiques, antiobésité de l'urine de dromadaire.
- Enquête sur l'activité neuroprotectrice et antioxydante du lait de dromadaire mélangé à l'urine de chamelle qui n'a jamais été mise en évidence.

Introduction

Introduction

The *Camelus dromedaries* known as the one-humped camel that is one of the most appreciated animals by the Arabian people and Bedouins in general and Algeria Saharan populations in specific. Globally, dromedary milk and its dairy products and its meat are widely consumed not only in Africa and Arab states, but also highly appreciated in Eastern and Central Asia, Latin America and even in Australia **(Alhadrami *et* Faye, 2022, Oselu *et al.*, 2022)**. In which, its adaptation to the harsh climatic conditions in its abundance areas is the cause and the origin of its usefulness and utility **(Ho *et al.*, 2021)**.

In fact, due to the its external and internal physiological properties, in addition to the uniqueness functionality of its organisms and metabolic pathways, the dromedary is able to maintain his biological needs and demands despite the very limited food sources and water quantities in its localization regions, especially in the hot periods **(Medjour, 2014).**

In point of fact, this animal is known by multiple properties that gives him the ability to adapt to the different condition of the Saharan and arid zones (Figure. 1).

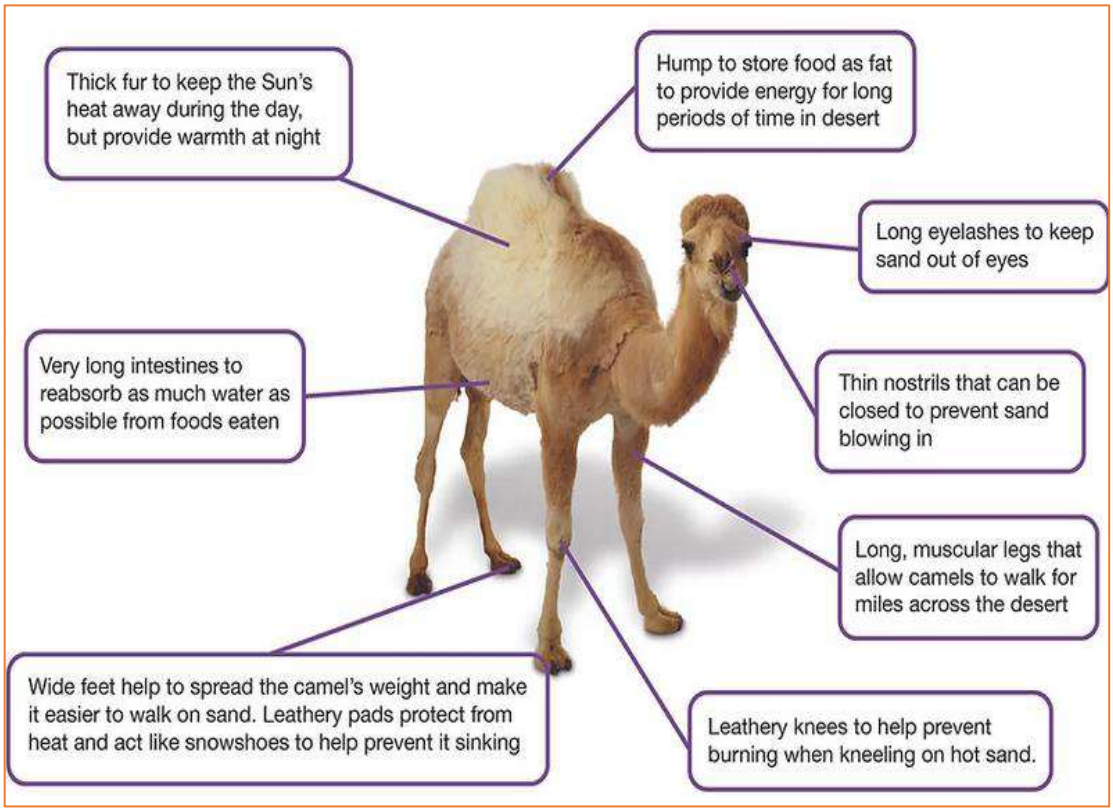

Figure 1. Adaptation proprieties of dromedary camel **(Cardellino *et al.*, 2004)**

In the recent decades, the importance productions of the *Camelus dromedaries* became widely noted in the literature, precisely the dromedary milk (DM). A product that has turn into one of the most studied subjects in the functional food domain **(Dubey *et al.*, 2015)**.

The reason refers to its precious nutritional value that the meat and milk components offer quite important biological functions to mankind **(Górska-Warsewicz *et al.*, 2019, Keyvan, 2021, McCormick, 2003)**.

Precisely, the dromedary plays a major role in supplying the inhabitants of the desert with a milk of good nutritional quality under extremely hostile conditions of temperature, drought and lack of pasture **(Medjour, 2014).** Therefore, dromedary milk had occupied a wide place in the dairy section researches on several levels due to its physical proprieties, chemical composition, functional characteristics, and its therapeutic utility **(Mostafa *et al.*, 2018 ; Rahmeh *et al.*, 2019 ; Swelum *et al.*, 2021 ; Minich *et al.*, 2022).**

Unfortunately, no precise information are perfectly available about the exact amount of dromedary (DM) milk production. In fact, in a desertic environment, it is difficult, if not impossible, to collect reliable data on the production of DM (**Cardellino *et al.*, 2004 ; Ho *et al.*, 2021)**. Mentioning that the production of this liquid is associated in a direct way to multiple factors including lactation length that from eight to eighteen months in general (longer durations on average than dairy cows under the same conditions), diet and herd management conditions playing an important role in the inconsistency of the data **(Cardellino *et al.*, 2004)**.

In addition to those factors, the breeding type play a key role in the reliability of the DM's production information. Effectively, the dromedaries are subjected to two breeding systems, traditional extensive type which is based on natural vegetation while the intensive breeding that is based on the use of feed supplements and concentrated diet, in which the milk production is count to be twice higher than in the extensive feeding system **(Field, 1979 ; Medjour, 2014).**

Moreover, according to several authors the improvement of feeding conditions prolongs the lactation period and increases the quantity of milk produced, sometimes reaching twice as much in the high food intake comparing to the milk production in the food poverty periods **(Bekele *et al.*, 2011 ; Musaad *et al.*, 2013 ; Oselu *et al.*, 2022)**.

Nonetheless, the food availability depending on the seasonal variability (climatic factors: heat, aridity) affects the dairy performance of this animal **(Medjour, 2014)**. As a matter of fact, a remarkable variation was noted in the different seasons which can affect more than 50% of this milk production that reaches its lower performance at the end of the dry season than in the rainy season **(Faye, 2004).**

Despite the lack of the exact productions amount of the DM, this milk count to be one of the most important rich foods in the desertic areas due to its specific and balanced

composition in all the nutrients **(Hammam *et* Agriculture, 2019**). Furthermore, the physiological, biological and metabolic proprieties of the *camelus dromedarius* influence the biosynthesis pathways of this biological fluid by producing unique and highly homogenized liquid rich in bioactive molecules **(Ali *et al.*, 2019)**.

From the general proprieties side, DM is characterized with a highly opaque white color, a consequence of its low fat and carotenoid composition **(Yagil *et* Etzion, 1980; Farah *et al.*, 1992 ; Kappelle, 1998).** It is combination of a slightly sweet, acidic and even salty taste with a bit sweet sharp taste comparing to other milk due to its pH that generally ranges between 6.2 to 6.8 **(Farah, 1993 ; Farah, 2004 ; Al Haj *et* Al Kanhal, 2010 ; Mal *et* Pathak, 2010; El Imam Abdalla, 2012 ; Parajapati *et al.*, 2012)**.

Additionally, this milk is less dense in a comparison with bovines' milk. Its freezing point goes from -0.57°C to -0.61°C, with a viscosity that is also lower than that of cow's milk **(Kamoun, 1995 ; Kherouatou *et al.*, 2003 ; Al Haj *et* Al Kanhal, 2010 ; Ghennam *et al.*, 2007 ; Faye *et al.*, 2008).**

The main chemical composition of dromedary milk includes the ash and total solids varying between 0.7 to 0.95% and 12.1 to 15%, respectively **(Konuspayeva, Faye, & Loiseau, 2009)**. Definitely the unique chemical functions of camel milk, refers to two main domains, the physiological and structural properties of camel cells as well as the organic feed that derived from the natural plants. The protein content generally varies between 3.5 to 4.5%, lipids consist of 3.07 to 5.50% and the lactose also varies between 3.4 to 5.6%, in which the more content in lactose the more this milk tends to the sweet flavor as reported by several authors **(Sawaya *et al.*, 1984 ; Zhang *et al.*, 2005 ; Zibaee *et al.*, 2015; Jilo *et* Tegegne, 2016 ; Pak *et al.*, 2019 ; Rahmeh *et al.*, 2019)**. Moreover, the lactose of this milk differs compared to the other mammals' milk lactose with its facility to be metabolized by the persons with lactose intolerance cases **(Hanna J *et al.*, 2001)**.

The lipidic part of DM showed a strong positive correlation with its protein content (**Parajapati *et al.*, 2012)**. However, only few works have been carried on the investigation of lipid material physicochemical and structural properties of this milk **(Hadddin, 2008)**.

Few studies conducted on dromedary milk's lipids characterization reported that the triglycerides represent 96% of total lipids. Moreover, among the lipids complex, the phospholipids in this milk consist of fatty acids mostly of C16:0, C18:1, C14:0, C16:1 and C18:0. Indeed it has being reported that the saturated fatty acids exist with an average of 59.33%, while the unsaturated fatty acids found with a percentage of 40.74 **(Ho *et al.*, 2021 ; Gorban *et* Izzeldin, 2001 ; Mohamed *et* Mustafa, 2013 ; Tasturganova *et al.*, 2018)**.

This particular distribution would largely explain the richness of this milk in lipids with a high melting point, therefore, in solid fatty substances at room temperature (25°C) **(Yagil *et al.*, 2000 ; Mohamed *et* Mustafa, 2013 ; Tasturganova et al., 2018)**.

Globally, dromedary milk proteins became the main focuses content of the researchers so far. Realistically, due to their nutritional contribution (source of essential amino acids) and their particular techno-functional properties, in which they were considerate to be more special comparing to other milk's protein (**Parajapati *et al.*, 2012),** this type of milk is a precious food for human health.

Additionally, according to their solubility in an acid medium, DM's proteins are divided as for the other milks, into two fractions: caseins and whey proteins (**Al Haj *et* Al Kanhal, 2010)**. The caseins precipitate at their isoelectric pH of 4.3, while the second category remain soluble in this considered pH level **(Wangoh *et al,* 1998)**.

Camel dromedary caseins are count mostly as phosphoproteins that represents the most abundant protein fraction, namely 73 to 81% of total proteins (**Al Haj *et* Al Kanhal, 2010)**. Those proteins are usually found in a micelles among them are molecular complexes made up of proteins, citrate, phosphate, calcium, magnesium, potassium and sodium (**El Imam Abdalla, 2012)**. Nevertheless, these proteins are divided into three groups: α-caseins (α-S1and α-S2), β-caseins and κ-caseins which were found to be twice lower in dromedaries' casein comparing to that of the bovines' (**Al Haj *et* Al Kanhal, 2010 ; Mal *et* Pathak, 2010; El Imam Abdalla, 2012)**.

On the other hand, the DM's whey proteins represent 18.5 to 27% of total proteins, with the exception of β-Lactoglobulin that was recorded to be absent in this milk type comparing to the cow's milk. For this reason, this milk is counted with a little or no allergic effects **(Sood *et* Sidhu, 1979 ; Mehaia *et al,* 1995** ; **Merin *et al.*, 2001 ; Al-Alawi *et al.*, 2011 ; El-Agamy *et al.*, 2009)**. However, some works have mentioned the presence of β-Lactoglobulin in a very minor content in some cases **(El-Agamy *et al.*, 2009 ; Hasni *et al.*,2023)**.

The DM's whey proteins consist also of α-Lactalbumin, camel serum albumin (CSA), immunoglobulins (Ig), proteose-peptones, lactoferrin, lacto-peroxidase and lysozyme **(Ochirkhuyag *et al.*, 1998 ; Kappeler *et al,* 1999 ; EL-agamy, 2000 ; Girard et *et al,* 2000).** The three Immunoglobulins were identified in this milk were the IgG, IgM and IgA with a predominance of G class **(EL-agamy *et al.*, 1996 ; EL-agamy, 2000)**. The IgG is an antibody, inhibiting its transfer via the placenta. Thus, it is offered to the newborns, precisely the colostrum **(Kinne *et al.*, 2001 ; El-Hatmi *et al.*, 2006)**.

In point of fact, it was reported by multiple researches that the IgG concentration reaches its maximal in the first lactation stage (first 7days of lactation) to protect the new born from different infections **(Kinne *et al.*, 2001 ; El-Hatmi *et al.*, 2006 ; Konuspayeva *et al.*, 2007 ; Makkawi *et al.*, 2019 ; El-Hatmi *et* Levieux, 2006 ; Al-Youcef *et al.*, 2012)**. Indeed, a huge part of the antimicrobial activity of DM is highly related to its important content in immunoglobulins Ig G, in addition to the presence of lactoferrin and lysozyme **(Ikeda *et al.*, 2000 ; Kinne *et al.*, 2001 ; El-Hatmi *et al.*, 2006 ; Benkerroum *et al.*, 2004 ; Redwan *et al.*, 2014)**. It was reported that the IgG of camel milk represent a very antiviral activity, particularly against rotaviruses **(El-agamy *et al*, 2012)**.

Moreover, the proteose peptone 3 (PP3) was characterized with an ability to inhibit the spontaneous lipolysis of milk and to stimulate the mitogenicity of hybridoma cells and the growth of bifidobacterial strains **(Mati *et al*, 1993 ; Girardet *et al.*, 2000 ; Mahmoudi *et al.*, 2016)**.

Besides the important functionality of the DM's proteins in their native structure, the released peptides from this protein either with *in vivo* or the *in vitro* digestion is considerate as a potential therapeutic choice for a several health problems **(Ebaid *et al.*, 2015).**

Effectively, until now multiple activities were founded to be ensured by the DM's peptides after their hydrolysis with pepsin, trypsin and chymotrypsin such as anti-oxidative, anti-hypertensive, anti-inflammatory and immunomodulatory activities **(Ebaid *et al*,. 2014 ; Ebaid *et* Ahmed, 2013)**. In addition to the anti-infectious and the anti-cytotoxic capacities that these peptides can guarantee to human body **(Salwa *et* Lina, 2010 ; Almahdy *et al.*, 2011 ; El-Fakharany *et al.*, 2012 ; Mal *et al.*,, 2006 ; Mona *et al.*, 2010)**.

Nevertheless, the vitamin content of DM is still under researches. So far, it was recorded that this milk contains less vitamin A, riboflavin, thiamin, folic acid and pantothenic acid than cow milk **(Algaithi *et al.*, 2022 ; Farah *et al.*, 1992; Faye *et al.*, 2019 ; Tastemirova *et al.*, 2022)**. On the other side, the pyridoxine, vitamin B12 and vitamin E concentrations are found in the same level between both dromedary and cow milks. Though, vitamin C, which is a strong anti-oxidant, was documented to be higher in DM (**El-Fakharany *et al.*, 2012 ; Mahmoud *et* Ahmed, 2013 ; Ebaid *et al.*, 2015 ; Zhao *et al.*, 2015)**.

The mineral part of DM is not well investigated. Only few inorganic elements were studied and their exact amount are given. The most researched minerals in camel milk were calcium, sodium, potassium, magnesium, chloride, phosphorus and zinc, which are found in a notable concentration **(Shamsia, 2009)**.

Globally, due to its anti-cancer and anti-diabetic properties, its high content of unsaturated fatty acids and low hypo-allergic effect, the fresh and fermented dromedary milk is nominated as an important natural healing source that can be used for medicinal purposes to several pathological conditions **(Konuspayeva *et al.*, 2009 ; Al-Juboori *et al.*, 2013 ; Zhao *et al.*, 2015)**.

Milk is not the only biological product that dromedary camel produce with this originality. Dromedary urine (DU) has also been used as a traditional healing ingredient by the Saharan Algerian population for long time. The vigorous and efficient recycling of urea and ammonia make this urine less or non-toxic **(Schmidt-Nielsen, 1979 ; Read, 1995)**.

Despite the popular utilization of the DU in the Arabian and Muslim cultures, a significant lack of data is recorded about this product. Indeed, few information are given about the exact composition and proprieties of dromedary urine. It was found that these urine are characterized by their wealth of potassium, phosphorus and magnesium, three ions known for their therapeutic effects and also the presence of proteins, triglycerides and cholesterol, generally absent in the human and animals urines **(Hasni *et al.*, 2015 ; Hasni *et al.* 2022).**

It was also documented that the urea, the urine principal component known for its toxicity, is present only at the state of traces in the camel's urines. Phenolic compounds such as polyphenols, yet the flavonoids were detected in a low notable amount **(Hasni *et al.*, 2022)**.

So far, it has been reported that this biological liquid has antibacterial, antifungal and antiviral proprieties due to some of its chemical composition **(Al-Yousef *et al.*, 2012)**. Moreover, **Agarwal and others (2009)** reported that it can be used to treat diabetic neuropathy.

Nevertheless, DU has also showed an antiplatelet ability, an inhibitory capacity against tumor and cancer cells *in vitro* such as the force to block the induction of Cyp1a1 which is a cancer activating gene **(Alhaidar *et al.*, 2011 ; Syed *et al.*, 2015 ; Alhaider *et al.*, 2017).** In fact, **Khorshid** and **Moshref (2006)** reported the anti-carcinogenic effect of this urine in different cancer types in rats.

The evaluation of the DU antioxidant activity by ABTS, DPPH, FRAP and PPM tests showed this urine expressed a very important antioxidant activity **(Hasni *et al.*, 2015 ; Hasni *et al.*, 2022)**. Nevertheless, it was found that this urine expresses a very weak hemolytic activity confirming its non-toxicity to the human body in case of consumption **(Hasni *et al.*, 2015).**

The dromedary urine is considered as a 'miraculous' product used in Prophetic Medicine, since the pre-Islamic era where camel milk and urine were used as drinking

medicine for different health problems **(Konuspayeva *et al.*, 2009, Omar *et al.*, 2010, Andreja *et al.*, 2015)**.

Despite the hard acceptance of the urine consumption, for the Bedouins population of arid and semi-arid regions, dromedary milk is not always consumed alone, but proportionally, mixed with a small amount of dromedary urine, which is count to be as an effective solution and candidate in the alternative medicine for multiple health disorders **(Kinne *et al.*, 2001 ; El-Hatmi *et al.*, 2006 ; Konuspayeva *et al.*, 2007 ; Al-Youcef *et al.*, 2012)**.

The two products DM and DU are classified as very important ingredient in the natural medicine or traditional pharmacology, which is the most used option for many people and in several cultures to fix internal and extrarenal health disorders (**Al Haj *et* Al Kanhal, 2010 ; Mal *et* Pathak, 2010 ; El Imam Abdalla, 2012)**.

A huge part of natural medicine is based on controlling enzyme activities relating to internal health disorders, especially the ones that become too common all over the world, such as diabetes and obesity, by either activating or inhibiting enzyme functionality causing those diseases **(Manna *et* Jain, 2015 ; Barr, 2018 ; Galicia-Garcia *et al.*, 2020)**.

Diabetes, one of the most common disease in the world that is a disorder of assimilation use and storage of sugars provided by food that occurs when the pancreas does not produce enough insulin (diabetes type I) or when the body is unable to use the insulin, it produces effectively, resulting in the accumulation of glucose in the blood known as diabetes type 2 (D T2) which is the most common form of the disease, that over time leads to serious damage to many parts of the body, especially nerves and blood vessels **(Fong *et al.*, 2004 ; Gudmundsson *et al.*, 2007 ; Chobot *et al.*, 2018 ; Quintero *et al.*, 2018 ; Saeedi *et al.*, 2019)**.

Although, the reasons for the onset of DT2 are still unknown. There are several important risk factors, including obesity which is defined as being a very significant accumulation of fat in the body affecting general health **(Mobbs, 2014 ; Chooi *et al.*, 2019 ; Blüher, 2020)**. It represents an evolved form of "overweight", a stage for which the harmful repercussions of adipose tissue on the body are less important. It is often estimated by the body mass index (BMI), **(Mobbs, 2014 ; Peltz *et al.*, 2010 ; Zhao *et al.*, 2018).** In which, the more the BMI increases, the more the risks of developing T2D increase in return. for these reasons, prevention of weight gain or weight loss in individuals with T2DM should be the primary therapeutic target **(Wilding, 2014 ; Franz, 2017 ; Apovian *et al.*, 2019)**. Both diabetes and obesity can be managed by controlling the activity of certain enzymes involved

in the digestion and metabolism of sugar and fats, such as α-amylase, α-glucosidase and lipase **(Tucci *et al.*, 2010 ; Zielińska *et al.*, 2020 ; Oluwagunwa *et al.*, 2021; Uddin *et al.*, 2022)**.

α-amylase and α-glucosidase enzymes are responsible for producing free mono-saccharides, especially glucose that moves from the intestines to the blood circulation. α-amylase acts on the hydrolysis of polysaccharides such as the starch into di and oligosaccharides that are subjected to hydrolysis into mono-saccharides by the α-glucosidase **(Kinne *et al.*, 2001 ; El-Hatmi *et al.*, 2006 ; Konuspayeva *et al.*, 2007 ; Makkawi *et al.*, 2019 ; gs; De Wit, 1998; El-Hatmi *et* Levieux, 2006 ; Al-Youcef *et al.*, 2012)**. One or both enzymes' functionality help decreasing of mono-saccharides concentration as the glucose in the internal system of the body, controlled by that hyperglycemia and T2D **(Kinne *et al.*, 2001 ; El-Hatmi *et al.*, 2006 ; De Wit, 1998; El-Hatmi *et* Levieux 2006 ; Al-Youcef *et al.*, 2012)**.

Conversely, Lipase is also classified as an important enzyme in managing obesity that increases the risk of developing DT2.Resistance to the action of insulin (insulin resistance) is frequently associated with excess body fat and is an early defect in the development of DT2. Adipose tissue metabolism is a major determinant of insulin sensitivity at the systemic level **(Kinne *et al.*, 2001 ; El-Hatmi *et al.*, 2006 ; Konuspayeva *et al.*, 2007 ; Makkawi *et al.*, 2019 ; De Wit, 1998 ; Al-Youcef *et al.*, 2012)**.

The natural medicine was oriented to the neuro-damaging diseases such as Alzheimer that has been known as the most common neurodegenerative disease and clinically characterized by progressive memory deficits and impaired cognitive function **(Moya-Alvarado *et al.*, 2016).**

The neurodegeneration disease Alzheimer can be caused by both, the hyperactivity of acetylcholine esterase (AChE) that hydrolyzes ACh with a turnover of 1,000 to 20,000 molecules per second (depending on the species) and the deterioration of the neural cells resulted by the free radicals' activity in the neural system **(de Oliveira *et al.*, 2012)**.

Beside AD, various pathologies and health disorders such as Parkinson's disease, cardiovascular diseases, immunological disorders, rheumatoid arthritis, diabetes or cancer and tumor cells developments, also renal diseases could be produced by one source oxidative stress **(Maddu, 2019)**.

As a matter, antioxidant substances have become a part of the that scientists research objectives for them in several areas such as food and nutrition, traditional medicine, phytochemistry, pharmacology in order to prevent the damages caused by the Reactive

Oxygen Species (ROS) that could be reached by a systematic mechanism controlling redox capacity or scavenging free radicals or reducing irons.

Until recently, several studies have been devoted to studying dromedary milk and its general proprieties on many levels, while no studies were dedicated to reveal the possible changes that can influence the physical and biochemical proprieties of the milk after being combined with dromedary urine despite its wide consumption and utilization as a healing product by the African Bedouins especially the Algerians.

The problem to be tackled in the thesis

To the best of our knowledge, there is no studies conducted on the importance of inhibiting certain enzymes like AChE using dromedary milk that has been used in the Bedouins culture like an alternative medicine, thus this study was carried out. Furthermore, until now, several types of research have been devoted to the antidiabetic and antiobesity effect of DM, but in literature there is no studies were conducted on the investigation of the ability of DM as mainly consumed by the Saharan tribes, mixed with DU against the enzymes relating to diabetic, obesity and AD.

Therefore, and given the highlighted pointes an important problem was posed, if these two heeling biological liquids were combined and what will be the biochemical characteristics of this formulation? Additionally, this formulation will have a similar therapeutic interest to the interests of the two separate liquids, or there will be a development of newly biological activities?

The aim of Thesis

The objectives of this research are first, to study the biochemical characteristics of the dromedary milk and urine separately and perform a formulation based on a mixture of these two biological fluids to find if there is an association and a combination of milk constituents with the constituents of the urine investigating the possible complementarity between their organic components. Moreover, to investigate some therapeutical forces that both liquids can offer independently and combined as the neuroprotective, antidiabetic, antiobesity and antioxidant activities.

Thesis structure

To reach the aims of the present study and to cover the highlighted point, this manuscript was organized in three major sections:

- The first was devoted to an introduction including an overall litterateur review about dromedary milk and dromedary urine, their proprieties and general benefits to the human health.
- The second part consisting of the methodological section illustrates a synthetic part on the used materials and the adopted as well as a general description of the followed protocols
- The third section concerned on all obtained results, followed by discussions. The thesis was ended up with a conclusion of all the work undertaken in addition to some perspectives for its continuity.

Novelty of the research

The present work reached novel finding, in which the objectives were never been reported that are as following: The characterization of the dromedary milk hydrolysates by peptic enzymes (*in vitro*) as by the FTIR and Zetasizer.

- The characterization of dromedary urine before and after *in vitro* digestion with FTIR, Zetasizer, HPLC.
- The determination and identification of some phenolic compounds in both dromedary milk and urine hydrolysates.
- This research highlights one of the undetermined capacities of the hydrolysates of camel milk which is the neuroprotective capacity by inhibiting and decreasing the activity of the acetylcholine-esterase (AChE) enzyme that causes the Alzheimer disease.
- The neuroprotective, antidiabetic, antiobesity activities determination of dromedary urine.
- Investigating the neuroprotective and antioxidant activity of the dromedary milk mixt with camel urine that was never highlighted.

Matériels et Méthodes

II. Matériel et méthodes

Le présent travail vise à déterminer les caractéristiques biochimiques et les intérêts thérapeutiques d'une formulation à base d'un mélange lait-urine de dromadaire. Par conséquent, la partie expérimentale est consacrée à l'étude de certaines caractéristiques physiques, chimiques et biochimiques du lait et de l'urine de dromadaire séparément et combinés à deux concentrations différentes. Par ailleurs, ce travail vise aussi à révéler et à évaluer quelques activités thérapeutiques que le lait et l'urine de dromadaire séparés ou mélangés peuvent offrir à la santé humaine en déterminant le pouvoir antagoniste ou synergique de leurs molécules respectives.

Pour atteindre l'objectif visé, l'expérimentation est divisée en trois grandes parties :

• La première partie consiste principalement à la caractérisation Physico-chimique et biochimique des échantillons étudiés ;

• la deuxième partie est consacrée à la digestion *in vitro* et à la caractérisation des hydrolysats des échantillons ;

• la troisième partie concerne l'évaluation *in vitro* des activités neuroprotectrice, antioxydante, antidiabétique et anti-obésité des hydrolysats des échantillons.

II.1. Présentation de la zone d'étude

L'étude est menée dans la partie désertique du sud-est de l'Algérie (nord-est du Sahara algérien), précisément entre Ouargla et Touggourt comme illustré sur la figure 2.

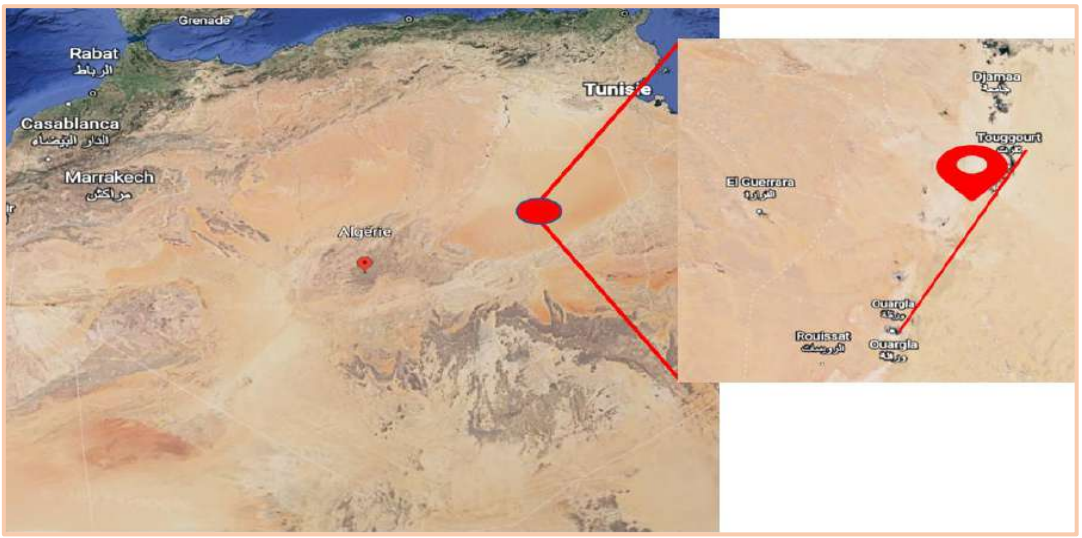

Figure 2. L'emplacement de la zone d'étude

II. 2. Materials biologiques

II. 2.1. Dromadaire

Selon les publications du département de recherche de Statista (2021), la population de dromadaires dans la zone d'étude représente environ 10% du cheptel national dont le nombre de chamelles est estimé à plus de 417 000 en 2018.

Les informations sur les chamelles d'où les échantillons sont prélevés sont consignées dans le tableau I.

Tableau I. Informations sur les animaux d'où les échantillons ont été prélevés

Sexe de l'animal	Femelles
État de santé	Saine
Stade de lactation	Multiple, non précis
Age (ans)	4 à 8
Mode d'élevage	Extensif

II.3. Méthodes

II.3.1. Échantillonnage

L'échantillonnage est effectué entre les mois d'août et d'octobre. Les précipitations à cette période ne dépassent pas en moyenne 9 mm. La température varie entre 17°C et 27°C la nuit et 39°C et 50°C le jour.

La collecte du lait et des urines de chamelles est effectuée tôt le matin entre 4h et 6h en présence d'un vétérinaire et avec l'aide d'un expert. Les échantillons sont recueillis dans des bouteilles stériles en verre ambré et sont immédiatement placés dans une glacière puis transportés au laboratoire.

Cette étude est réalisée sur le lait de dromadaire (DM), les urines de dromadaire (DU) et deux mélanges de DM avec DU à deux concentrations différentes :

- Un mélange lait- urines avec un rapport de 9 :1 (v : v) (DMU1).

- Un mélange lait- urines avec un rapport de 8 :2 (v:v) (DMU2).

II.3.2. Caractérisation des échantillons

Les échantillons prélevés et les deux formules ont fait l'objet des tests pour les caractérisées physiquement, chimiquement et biochimiquement comme montré dans la figure 3.

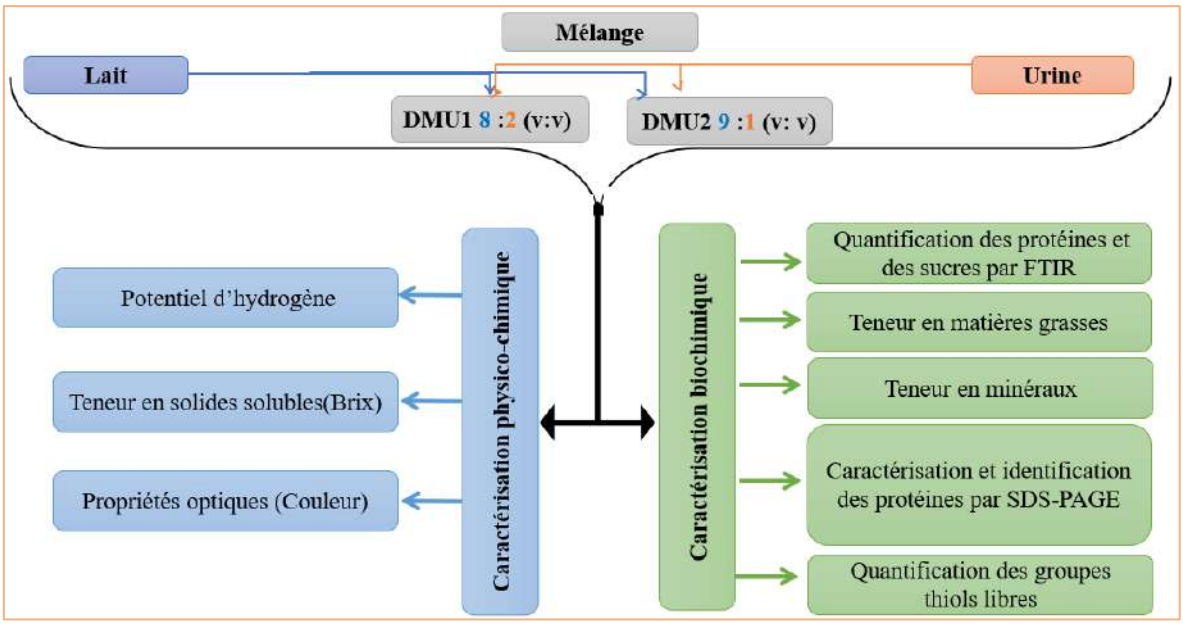

Figure 3. Organigramme des analyses physicochimiques et biochimiques des échantillons

II.3.2. Caractérisation des hydrolats et évaluation de leurs effets thérapeutiques

Le lait, l'urine et les deux mélanges sont soumis aux conditions de digestion *in vitro* en présence de la pepsine et de la trypsine. Les hydrolysats obtenus sont caractérisés et certaines de leurs activités biologiques sont évaluées (Figure 4).

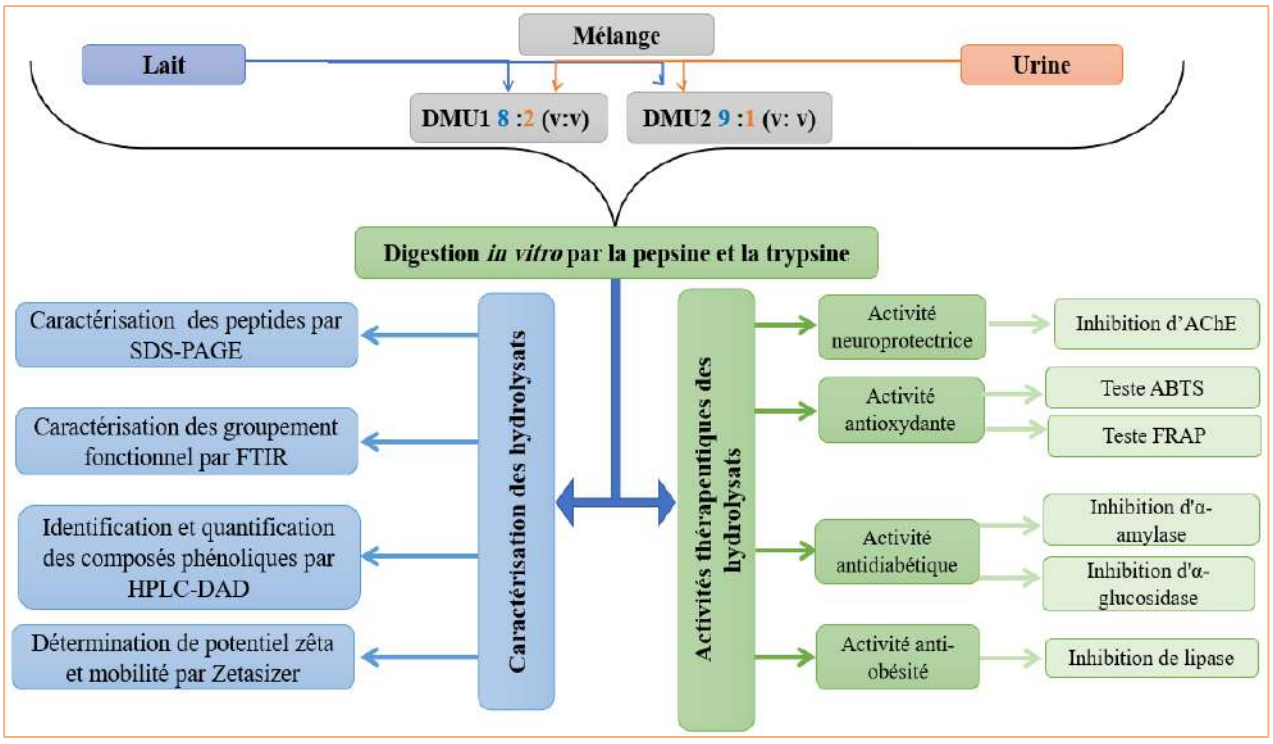

Figure 4. Organigramme des analyses biochimiques et des activités biologiques des hydrolats.

Materials

&

Methods

II. Materials and methods

The present work aimed to determine the biochemical characteristics and therapeutic interests of a formulation based on a mixture of dromedary milk-urine. Therefore, the experimental part was devoted to investigate some physical, chemical, and biochemical characteristics of both dromedary milk and urine separately and combined at two different concentrations. Moreover, to reveal and evaluate some therapeutical foresees that dromedary milk and urine can offer to the human health alone and mixed in order to determine the antagonist or the synergic role that the urine molecules can play to the milk compounds.

Based on the research aim, the experimental section was divided to three major parts:

- The first part consists mainly on the characterization of the studied samples at the Physico-chemical and the biochemical levels.
- The second part was devoted to the *in-vitro* digestion and characterization of the samples' hydrolysates.
- The third part focused on the evaluation of *in-vitro* neuroprotective, antioxidant, antidiabetic, and antiobesity activities of the samples' hydrolysates.

II.1. Presentation of the study area

The study was conducted in the desertic south-eastern part of Algeria (northern-east Algerian Sahara), precisely between Ouargla and Touggourt as illustrated in figure 2.

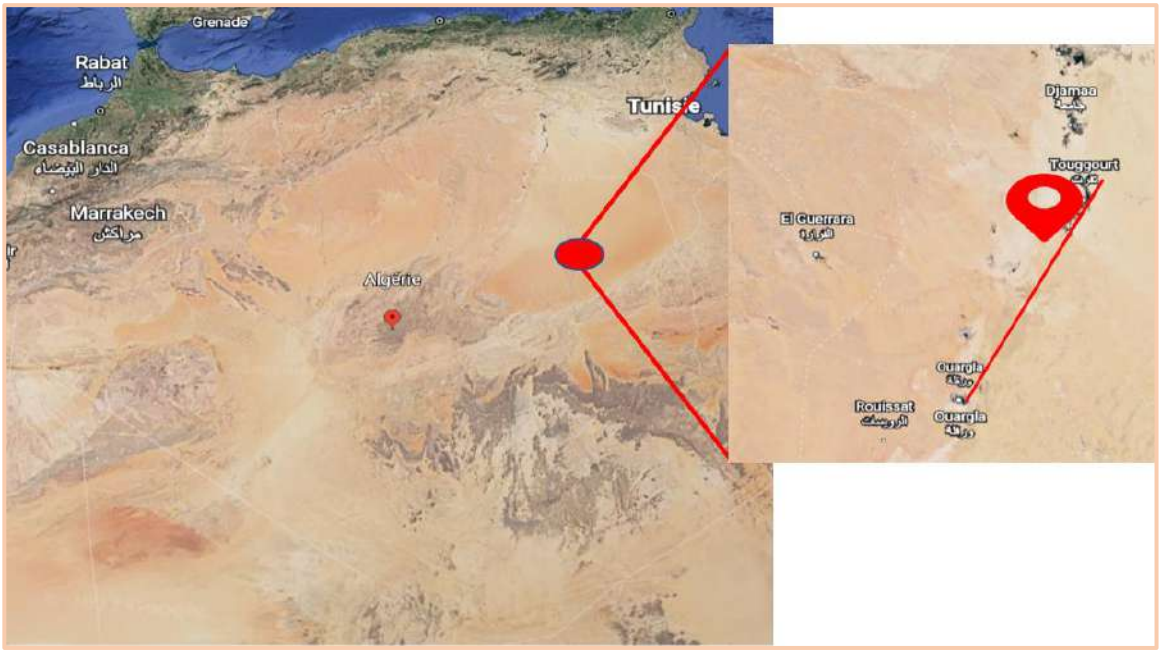

Figure 2. Map showing the location of study area

Both states have a typical warm desert climate with very long and extremely hot summers and short mild winters. The climate is very dry all year with an annual rainfall of around 45 mm **(Kinne *et al.*,2001 ; El-Hatmi *et al.*, 2006 ; Levieux, 2006 ; Konuspayeva *et al.*, 2007 ; Al-Youcef*et al.*, 2012 ; Makkawi *et al.*, 2019).** The geographic coordinates of both areas are cited in table I.

Table I. Geographic orientations of the study areas.

Region	Latitude	Longitude	Altitude
Ouargla	31°56′57″ North	5°19′30″ East	138 m
Touggourt	33°06′18″ North	6°03′28″ East	72 m

II. 2. Biological materials

II. 2.1. Dromedary

The dromedary population in the study area represent about 10% of the national herd, which was estimated to be more than 417 thousand in 2018 as published by Statista Research Department in 2021.

Dromedary information from which samples were taken is cited in the following table:

Table II. Information of the *Camelus dromedarius* from which samples were collected

Sex of the animal	Females
Health status	Disease free
Lactation Stade	Multiple, unspecified
Age (years)	4 to 8
Feeding	Only on the Saharan plants with free access to wells and oases water

II. 2. 2. Enzymes

In order to determine the highlighted biological activities of the tested samples four main enzymes were used:

- The acetylcholine esterase (AChE): from *Electrophorus electricus* (electric eel), Type V-S, lyophilized powder, ≥1,000 units/mg protein (Sigma).
- The α-amylase: from porcine pancreas, type VI-B, ≥10unites/mg solids (Sigma).

- The α-glucosidase: from *Saccharomyces cerevisiae*, type I, ≥10unites/mg (Sigma).
- The pancreatic lipase: from porcine pancreas, type VI-S, ≥20000units/mg protein (Sigma).

II.3. Methods

II.3.1. Sampling

The sampling was carried out between the months of August and October. The rainfall in this period does not exceed an average of 9 mm. The temperature varies between 17°C and27°C at night, and from 39°C to 50°C at day.

The Collecting of dromedary milk and urine was performed at the early morning (4 am - 6 am), with the presence of a veterinary and with the aid of a professional expert. The samples were collected in sterile amber glass bottles and were immediately cooled and transported to the laboratory for further analysis.

This study was executed on dromedary milk (DM), dromedary urine (DU), and two mixtures of DM with DU at two different concentrations as following:

-Dromedary milk incorporated with urine with a ratio of 9:1 (v: v), respectively (DMU1).

-Dromedary milk incorporated with urine with a ratio of 8:2(v: v), respectively (DMU2).

II.3.2. Samples characterization

II.3.2.1. Physical and chemical characterization

II.3.2.1.1. Hydrogen potential

The Hydrogen potential (pH)is one of the important characteristics that gives a general idea of the acidity state of the product. It indicates the concentration of hydrogen ions in a substance or solution and measures the degree of activity of the hydrogen ion inside it **(Peech, 1965 ; Tanford, 1970 ; Caenn *et al.*, 2017)**. Knowing by that the possible interactions that can accrue to the milk after being combined with urine.

From a solution consisting of 10 ml of the fresh samples, the pH was determined at the room temperature with a pH meter (Thermo-Scientific Orion Star A111, Indonesia) that was calibrated before each measurement with a standard buffer at 25°C **(McMillan *et* Cameron, 2004 ; Scientific, 2014)**.

II.3.2.1.2. Total soluble solids content (Brix)

Total soluble solids (TSS) of the tested samples were measured using refractometer, which determines the refractive index of light from a solid or liquid matrix **(Basak *et al.*, 2022)**. This index is observed by the deflection of a light beam depending on the nature of the medium in which it propagates. The angle of the beam deviates according to the rate of soluble dry matter in the medium, the higher the concentration of soluble dry matter, the greater the refraction **(Hoffmann, 2001 ; Sun *et* Fan, 2015 ; Rawle, 2017)**. The refractive index is a well-known application in determining the soluble content in food products, a method that can be used for determining the TSS in milk, urine and the two combinations so a comparison between the samples can be made.

At the room temperature, a suitable amount of 2 to 3 drops of the fresh samples was placed in the prism-plate of the Digital-Bench- Refractometer (model 10430 with porx reading range 0-30 Bausch and Lomb Co. California, USA) that was cleaned and calibrated before each measurement with distilled water. The rate of the soluble solids appearing on the device screen is recorded as Brix **(Pang *et al.*, 2016 ; Rawle, 2017)**.

II.3.2.1.3. Optical properties (Color)

The color of an aliment in general and of milk in specific is considered as an intrinsic signal that motivates its consumption. The color of the milk depends on its components and the homogenization of its molecules. The introduction of external elements to this homogeneous suspension can causes a remarkable changing on its general optical proprieties **(Foto's *et* De Gusseme, 2014 ; Smirnova *et al.*, 2020 ; Al-Hilphy *et al.*, 2022)**. Thus, analyzing the DM alone and after being combined with DU color parameters was necessary.

As described by **Milovanovic *et al.* (2020)**, the Konica Minolta (CR-400, Japan) colorimeter was used to evaluate the three-color values L*, a*, and b* of the studies samples. The L* value is an indicator of whiteness-blackness varying from 0 (black) and 100 (white), the a* parameter designating redness-greenness with values that goes from -60 (green) to +60 (red), and the b* character that refer to the blueness and yellowness, indicator confined between -60 (blue) and +60 (yellow).

A standard white ceramic calibration plate was used for the calibration of the device which was cleaned before each measurement. The registration of the color parameter was carried out at 5 different points of each sample with three repetitions (n=3) at the room temperature, where the standard white plate values L*= 93.87, a* = 0.55, and b* = 5.13**(Milovanovic *et al.*, 2020)**.

II.3.2.2. Biochemical characterization

II.3.2.2.1. Proteins and sugar quantification by FTIR

The chosen technic for proteins and sugar quantification in the tested samples was the Fourier Transformation InfraRed spectroscopy (FTIR). A technique with many advantages. It is a fast way that saves a lot of time and requires only a small amount of sample. Plus, this method requires no preparation steps. FTIR method allows the detection of the interaction between matter and infrared radiations that can trigger and give rise to the vibration of specific molecular bands **(Nicolet *et* All, 2001 ; Baker *et al.*, 2014 ; Hofko *et al.*, 2017)**. This technique can be used as a fingerprint for chemical compounds which mean a good way for materials characterization and identification by giving the absorption and emission spectrum for molecular and atomic analysis **(Jones et Hench, 2005 ; Titus *et al.*, 2019 ; Wen *et al.*, 2021)**.

For the proteins and sugar quantification by FTIR, a volume of 10µl of each sample was placed in to the ATR crystal surface of a Bruker Tensor 27 spectrometer equipped with a DLA TGS detector (Bremen – Germany). At mid-infrared area with a band interval of 4000-600 s^{-1}, the spectrums were collected with a resolution of 4cm^{-1} for the FTIR scanning recording (accumulation of 16scan/spectra). The data procuration was performed with the OPUS program (7.2 version for Windows from Bruker GMBH). The Distillated water was used as a background to eliminate water bands absorbance and the samples traces were eliminated after each injection with deionized water and ethanol (80% v: v) **(Nicolaou *et al.*, 2010 ; Baddela *et al.*, 2016 ; Algethami *et al.*, 2020 ; Rachah *et al.*, 2021)**.

In the present study, FTIR was employed to determine the concentration of proteins and sugar in the DM, DU, DMU1 and DMU2 based on the bands absorbance that allowed to draw a calibration curve. The proteins quantification was carried out by the detecting of the amid I band of the secondary structure of proteins that produce a peak between 1600 $cm^{-1}$and 1700 cm^{-1} area as shown infigure 1**(Usoltsev *et al.*, 2019)**. The Bovine Serum Albumin (BSA) protein was chosen for the calibration curve obtention with a concentration ranging from 1mg/ml to 10 mg/ml (Figure 3).

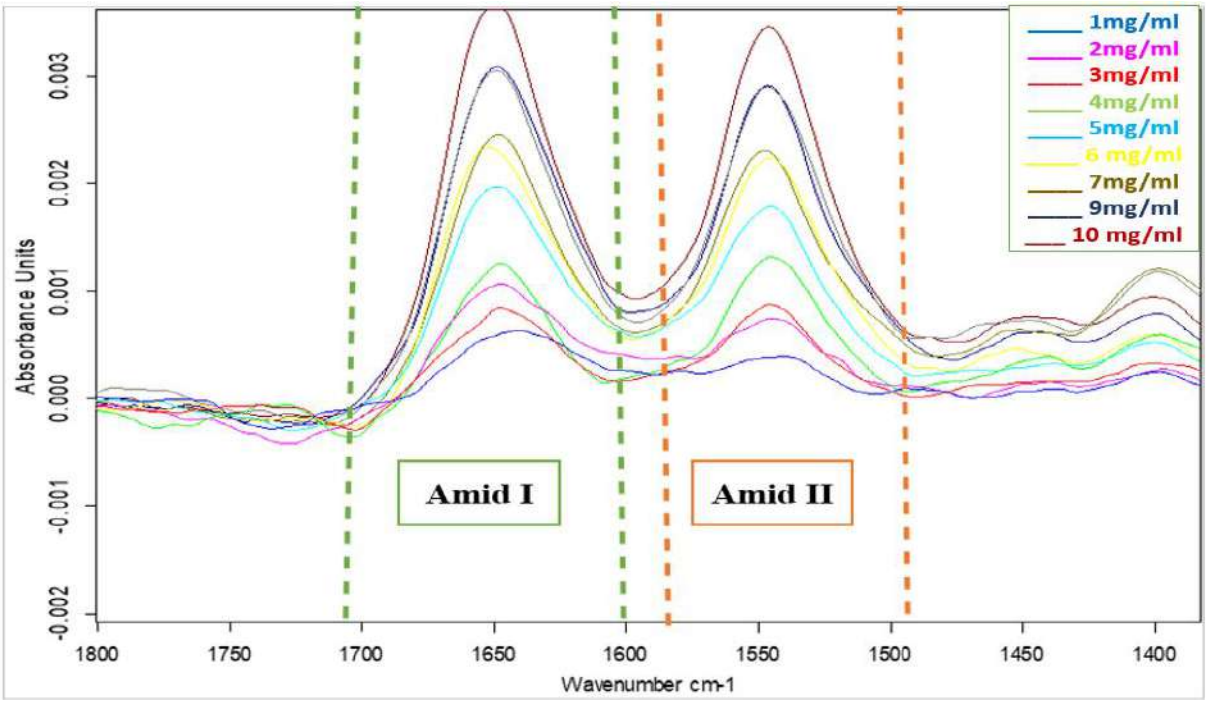

Figure 3. FTIR spectrum of the BSA standard protein in the Amid I with integration area of 1710.7 cm^{-1} – 1604.1 cm^{-1}.

Each concentration from the used standards was scanned for three times and the average of each concentration was taken for drawing the calibration curve. After each spectrum acquisition, ATR correction was performed (Figure 3 and 4). The calibration curves from where the calculation of the protein and sugar concentration are representing in Figure 3, and 4, respectively.

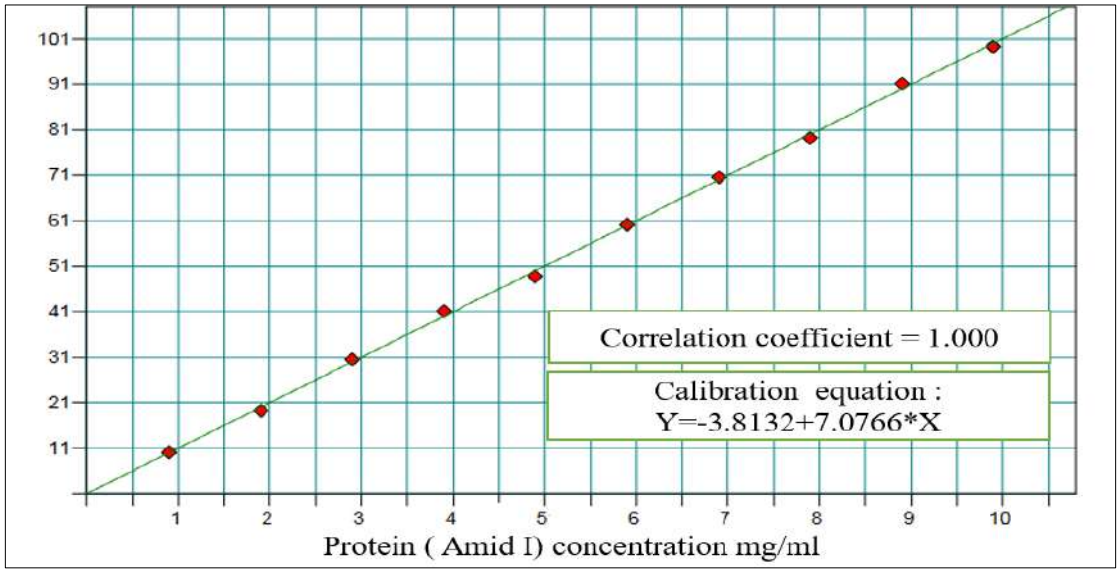

Figure 4. Calibration curve of the amid I of the standard protein (BSA) obtained by the OPUS program (7.2 version for windows from BRUKER GMBH).

The sugar quantification was obtained by the absorbance spectra between 1180 and 960 cm^{-1}, precisely at 1035 cm^{-1} that corresponds to the glucose concentration **(Nybacka, 2016)**. Pure glucose (Sigma-Aldrich) was the reference for the sugar detection with a concentration goes from 1mg/ml to 6mg /ml (Figure 5).

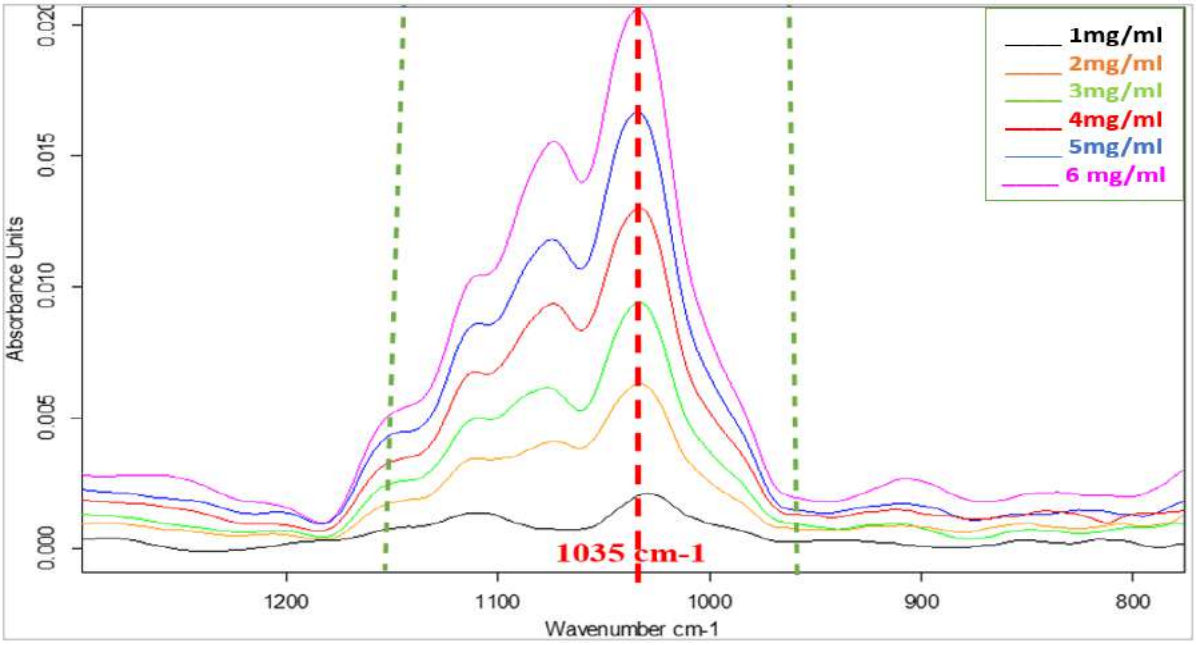

Figure 5. The FTIR spectrum of sugar standard with integration area of 1180 and 960 cm-1, precisely at 1035 cm-1 that corresponds to the glucose concentration.

Calibration curve of the pure glucose that was applied for the quantification of the sugar content in the four tested samples is demonstrated in figure 6.

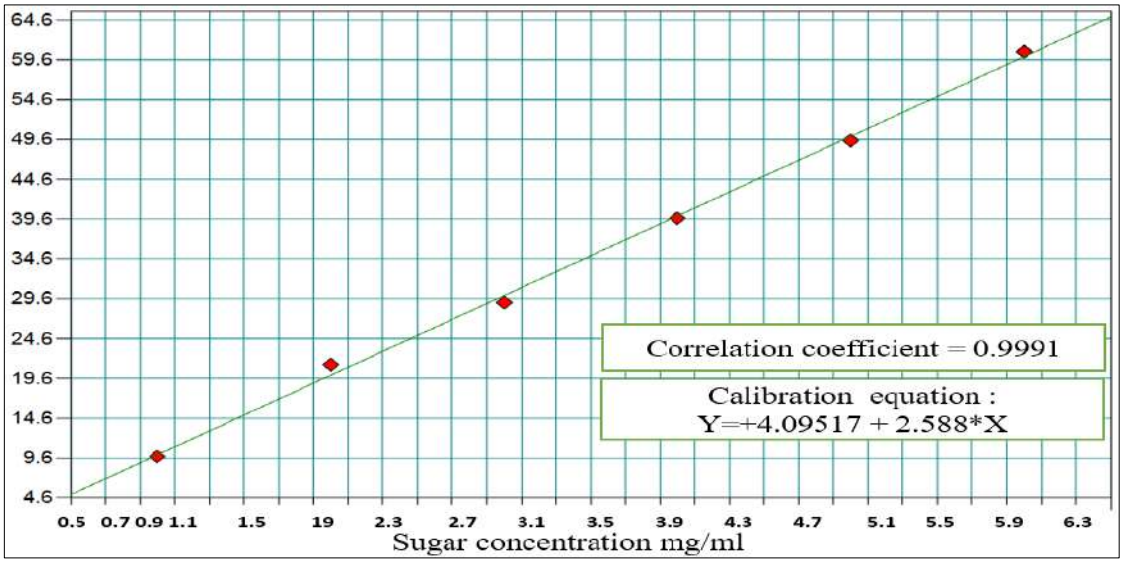

Figure 6. Calibration curve of the sugar (Glucose) obtained by the OPUS program (7.2 version for windows from BRUKER GMBH).

II.3.2.2.2. Fat Content

The quantification of the fats was performed by the GERBER application, where 11 ml of samples with milk (DM, DMU1, and DMU2) are added to 10 ml of sulfuric acid with a density of 1.5 g/ml. This step aimed to dissolves the proteins and the non-fat constituents in order to be eliminated. Next, 1ml of iso-amyl alcohol was added to the butyrometer for the separation of fatty material. The sample, acid and alcohol mixture was stirred to facilitate the dissolution of the various components of the milk and centrifuged for 10 min. The butyrometer was immersed vertically in a water bath brought to 70° C for 5 min to promote the separation between the dissolved lipid phase and other components **(Kleyn *et al.*, 2001)**.

The fat content (FC) was calculated using the following formula:

$$\mathbf{FC = B - A}$$

Where A and B are the readings taken at the lower and upper ends of the FC column, respectively.

II.3.2.2.3. Minerals content

Calcium (Ca), Sodium (Na), Potassium (K) and Chloride (Cl) in the DM, DU, DMU1and DMU2 were quantified using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). A very sensitive, specific and rapid technique that requires a few amounts of the sample with a multi-element' quantification in the desired matrices **(Bulska *et* Wagner, 2016 ; Wilschefski *et* Baxter, 2019)**. An instrumental technique of analysis based on the separation, identification and quantification of the constituent elements of the sample according to their mass. It is based on the coupling of a plasma torch generating ions and a quadrupole mass spectrometer which separates these ions in mass. The analysis of samples by ICP-MS can be divided into introduction-nebulization, ionization, mass separation, and detection steps **(Greiner *et al.*, 2015 ; Nageswaran *et al.*, 2017 ; Al-Hakkani, 2019 ; Jiménez-Lamana *et al.*, 2020)**.

Firstly, the sample is placed in solution and an automatic sample changer coupled to a peristaltic pump introduces the solution into a vaporization chamber where the nebulizer transforms it into a liquid aerosol composed of micro-droplets of a few µm using argon gas. The formed aerosol is sent to an argon plasma torch at very high temperature (between 6,000 and 10,000°C), sufficient to vaporize, dissociate, atomize and completely ionize most elements. In this step the ionization happened **(Sharp *et* O'Connor, 2006 ; Jagodić *et al.*, 2021)**.

A differential vacuum system accelerates the ions from the plasma to an array of electrostatic lenses which extract the positively charged ions and transport them to a quadrupole mass filter. The principle of the spectrometer is based on the separation of the elements according to their charge and their mass. The four cylindrical bars that make up the spectrometer are separated into two opposite pairs and subjected to direct (DC) and alternating (AC) current. The two pairs have opposite DC and AC voltages of the same magnitude and opposite sign. In the plane formed by the positive pair, the light ions are deflected too much and hit the bars. The ion to be analyzed and those with a higher mass remain between the two bars. In this plane, the quadrupole acts as a high-pass filter. In the plane of the negative pair, it is the heavy ions that are deflected, which is equivalent to a low-pass filter. By combining these two filters, only ions with the desired m/z (mass/charge) ratio will be transmitted to the detector **(Batey *et al.*, 2005 ; Al-Hakkani, 2019 ; Wilschefski *et* Baxter, 2019 ; Laur *et al.*, 2020)**.

The detection part is carried out using an electron multiplier with discrete dynodes. For the detection of positive ions, a series of dynodes is subjected to a negative voltage of a few thousand volts. This positive ion causes the emission of one or more secondary electrons which again strike the wall of the second dynode. At the end of the series of dynodes reach a collector equipped with a preamplifier. The signal is translated into a number of pulses (number of strokes), a computer interface ensures the transfer of data during the process. For a given isotope, the number of ions measured makes it possible to directly calculate the concentration of the element analyzed using quantitative and qualitative recording processing software **(Batey *et al.*, 2005 ; Al-Hakkani, 2019 ; Wilschefski *et* Baxter, 2019 ; Laur *et al.*, 2020 ; Tian *et al.*, 2022)**.

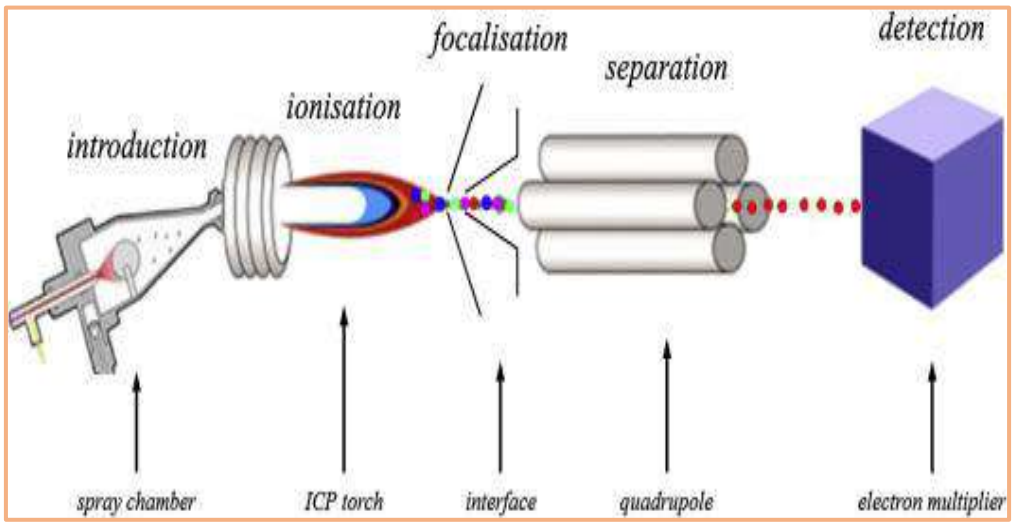

Figure 7. Demonstration of the ICP-MS principal.

In our case, 1ml of each sample was introduced to a solution combined of 0.1ml of HNO_3, 8.8 ml of DW and a volume of 0.1 ml of the internal standards. The hall mixture was well homogenized and introduced to the equipment with a flow rate of 1ml/ min and with an integration time that was calculated by multiplying the sweeps number by the dwell time which was 10 ms for Na and K and 20 ms for Ca and Cl (IT = 350 ms for Na and K and 700 for Ca and Cl). Three replicates were conducted for each sample and the average was recoded. The Perkin Elmer's Elan6000 was the used interment for the ion detection **(Zwierzchowski *et* Ametaj, 2019)**.

II.3.2.2.4. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE is a widely used technique for separating mixtures of proteins, based on their size and nothing else that was first described by **Laemmli (1970)**. SDS, an anionic detergent used to produce equal charge across the length of proteins that have been linearized **(Rothe, 2000 ; Gudiksen *et al.*, 2006)**.

Firstly, proteins are loading into a polyacrylamide gel in which an electric field is applying; the SDS-coated proteins are then separated. The electric field acts as the driving force, directing the SDS-coated proteins towards the anode with larger proteins moving slower than smaller proteins. In order to identify proteins by their size, proteins standards of known sizes are loaded in parallel with the samples and subjected to the same conditions **(Rothe, 2000 ; Righetti, 2005 ; Gudiksen *et al.*, 2006 ; Pincus, 2012)**.

SDS-PAGE method was carried out in order to identify the milk samples' proteins and to investigate the possible molecular and structural modifications that may accrue to the DM proteins by adding the DU.

A volume of 20µl of a solution prepared by mixing 200µl of each sample with 200µl of the sample Tris-HCl buffer at pH 6.8. A buffer contains sodium dodecyl sulphate (SDS) and β-mercaptoethanol; the thiol reducing agent. The mixture was then boiled for 5 min then left to cool down, to be then injected to the gel halls. The proteins separation was illustrated on gradient acrylamide gel of 7.5% - 20% so all ranged protein can be visualized. The separation was illustrated at a constant current of 40mA/gel (Bio-Rad Laboratories, Inc., Richmond, CA, USA). The β-mercaptoethanol-bromophenol blue was used to dye the samples. Moreover, the electrophoresis gel was stained with the use of the Coomassie brilliant blue for 24h and distained with methanol solution (50%) for four hours **(Muguruma *et al.*, 2012)**.

The determination of the interest proteins molecular weight was carried out by migrating in parallel (under the same conditions) standard proteins of a known molecular weight.

II.3.2.2.5. Protein oxidation test

Changing proteins medium by modifying its compositions or conditions may lead to serious damages to the proteins' conformation, structure and functionality **(Alberts *et al.*, 2002 ; Sun *et al.*, 2004)**. One of the most possible consequences caused by that is protein oxidation (PO) which can be measured by detecting the products resulted from the oxidation process. This process can touch the proteins on several levels, such as oxidation of aromatic moieties, glycoxidation, carbonylation, nitration and oxidation of amino acids sulfur-containing mainly cysteine's sulfhydryl group and methionine' thioether group due to their sulfur atoms. Sulfhydryl group known also by thiol group, are made up from the bonding of a sulfur atom with hydrogen **(Zhang *et al.*, 2013 ; Boguszewska-Mańkowska *et al.*, 2015 ; Ahmed *et al.*, 2016 ; Kehm *et al.*, 2021 ; Domínguez *et al.*, 2022)**. The free sulfhydryl group generated from protein amino acids' oxidation are easily reacted with the 5.5′-dithio-bis (2-nitrobenzoic acid) or DTNB (the Elman reagent) to produce TNB–thiol-activated; species characterized with their high coloration that can be detected spectrophotometrically (Figure 8) **(Gergel *et* Cederbaum, 1997 ; Badyal *et al.*, 2001 ; Tawfik, 2002 ; Rudyk *et* Eaton, 2014)**.

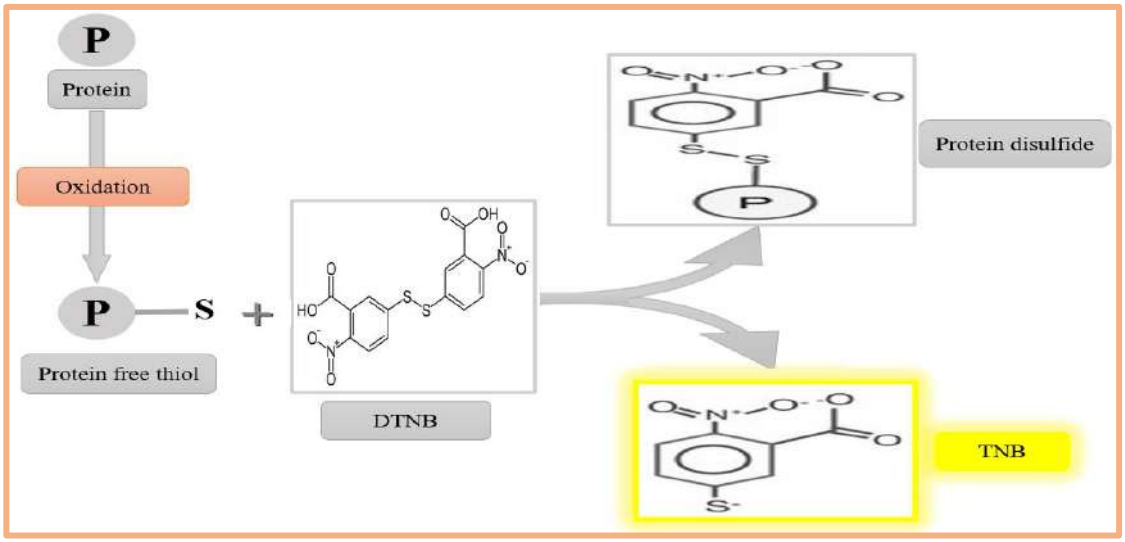

Figure 8. Principal of the spectrophotometrically measurement of the free thiol group content generated by protein oxidation with the DTNB (Ellman assay).

Based on the Ellman assay, the measurements of thiol and disulfide bond contents were performed to detect the milk PO caused by DU addition. A volume of 0.2ml of each sample of DM, DMU1 and DMU2 was introduced to 4ml of Tris-EDTA buffer (pH 8), followed

by the addition of 0.08ml of DNTB solution (10mM). After incubation for 15-20min at the room temperature, the mixture was centrifuged for 10min at 3000g. The supernatant absorbance was measured at 412nm. Each sample was accompanied with a blank that was prepared in the same condition and in parallel with 0.03 ml of each sample and 4ml of the buffer excluding the DNTB solution **(Mahcene *et al.*, 2021)**. The sulfhydryl groups (-SH) concentration was determined with the application of the general formula which is:

$$-\mathbf{SH} = \frac{(\mathbf{A} - \mathbf{B} - \mathbf{volume\ of\ the\ sample\ blank}) * (\frac{\mathbf{volume\ of\ the\ solutione}}{\mathbf{volume\ of\ the\ sample}})}{\mathbf{TNB\ molar\ extinction\ coefficient}}$$

By the introduction of the TNB molar extinction coefficient which is 14,100$M^{-1}cm^{-1}$ and 4.28ml of the solution. According to the following equation, the free thiol group's results were reported in micromoles per liter (µmol/l).

$$-\mathbf{SH} = (\mathbf{A} - \mathbf{B} - \mathbf{0.03}) * \mathbf{1.52\ mM}$$

Where the A indicates the sample absorbance and B is the blank absorbance.

II.3.3. *In vitro* digestion test

Since most of the therapeutical forces of DM are related to the peptides released from the milk protein digestion, the digestion process was caried out only on the protein part using proteolytic enzymes, pepsin and trypsin. Nevertheless, in order to be exposed to the environment of the stomach and intestines (pH and enzymes), DU samples were also hydrolysates as the milk samples.

The *in vitro* hydrolysis of the samples was performed according to the method described by Egger *et al*., (2019) in two major steps. Firstly, pepsin was added to the substrate in 1:100 (W/W) ratio after adjusting the pH to 1.5 with HCl. The mixture was rotated at 70 rpm for 2 h at 37°C. The enzyme inactivation was accomplished by heating the mixture at 95°C for 10 min. After letting the mixture cold down, the medium pH was readjusted to 7.5 with NaOH and trypsin was added in a 1:100 (w/w) ratio (enzyme/ substrate). The mixture was re-incubated at 37°C for two hours in a continuous agitation (70rmp). The tubes were placed in boiling water again to block the trypsin activity. Finally, the resulted solution was centrifuged at 4500 rpm for 15 min at room temperature. The supernatant was filtrated using 45µm membrane **(Egger *et al.*, 2019)**. The hydrolysates were stored at -80°C until analysis.

II.3.4. Hydrolysates characterization

The hydrolysates of DM (DMH), DU (DUH), DMU1 (DMUH1) and DMU2 (DMUH2) were subjected firstly to the SDS-PAGE assay on two gradients to ensure the success of the digestion process and to have general visualization on the released peptides; then all the hydrolysates were characterized by the FTIR, Zetasizer and HPLC.

II.3.4.1. Hydrolysates' SDS-PAGE

The SDS-PAGE procedure was accomplished after the *in vitro* digestion of all the samples, as described previously. For the visualization of high-MW proteins (nondigested), proteins samples were separated with 7.5-20% (w/v) acrylamide gradient slab gel. The generated peptides were visualized on a 7.5-30% gradient. The constant current in both cases was 40mA/gel (Bio-Rad Laboratories, Inc., Richmond, CA, USA **(Muguruma *et al.*, 2012)**.

II.3.4.2. FTIR characterization

In order to detect the possible interactions between the functional groups of the DMH and DUH that can appear in the patterns of the DMUH1 and DMUH2, FTIR scanning of all the hydrolysates samples was carried out. Shortly samples were directly compressed on the ATR crystal surface to be scanned at a band interval of 4000-600s^{-1}, where distilled water was used as a background. The specters analysis was carried out with the Bruker Tensor 27 spectrometer equipped with a DLA TGS detector (Bremen - Germany). The OPUS program (7.2 for Windows from Bruker GMBH version) was used for the data acquisition.

II.3.4.3. Phenolic compounds detection by HPLC-DAD

Many therapeutical activities offered by either dromedary milk or urine are claimed to be related to the possible existence of secondary metabolites that comes from the browsed plants by this animal, knowing that dromedary consumed essentially desertic medicinal plants. Therefore, High Performance Liquid Chromatography (HPLC) was carried out to analyze quantitively and qualitatively some phenolic compounds depending on the retention time relating to the calibration standard.

From each hydrolysates sample 1 ml was inserted into the HPLC equipment (HPLC-DAD, Shimadzu Corp., Kyoto, Japan) for phenolic compounds analysis. The separation was carried out at 40°C on a reversed-phase column. Distillated water with 0.1% (V/V) of acetic acid was used as the mobile phase A and the mobile phase B was composed with 0.1% (V/V) of acetonitrile and acetic acid. The flow rate was 1ml/ min and the gradient elution was 63min;

starting with 10% B from 0-2min than10% to 30% B beginning with 2 to 27min; than from 30% to 90% B from 27 to 50min and 90% to 100% B from 51 min till 60 min. The detection of the target compounds was at 254-356 nm **(Kayacan *et al.*, 2020)**.The parameters description of the HPLC equipment used for phenolic compounds dosage in this study are presented in table III.

Table III.HPLC equipment' parameters.

Parameter of HPLC system	Description
Pump	LC-20AD
Autosampler	SIL-20A HT
Oven	CTO-10ASVP
Degasser	DGU-20A5R
Communication module	CMB-20 A
Diode array detector	SPDM20A DAD (Shimadzu, Japan)
Wavelengths	278, 325, 236, 254 and 517 nm
Column	C18 column5 µm15 X 3.9 mm / 15 X 4.6 GL sciences Inertsil ODS-3 (25 cm× 4.0 mm, GL sciences, Tokyo, Japan
Column temperature	40°C

II.3.4.4. Zeta-sizer characterization

The zeta potential represents the electrical charge that a particle acquires due to the cloud of ions which surround it when it is in suspension or in solution. Indeed, in a liquid, particles surround their-self with ions organized in a "double electric layer" **(Xie *et al.*, 2011 ; Marsalek, 2014)**. Some of the ions' cling to the particle, thus forming a layer of adherent ions called the dense layer, the other part of the ions forms an unbound so-called diffuse layer. The "shear plane" delimits these two layers. It is the potential difference between the dispersion medium and the potential at the shear plane that defines the zeta potential. This potential represents the measure of the intensity of repulsion or electrostatic attraction between particles **(Selvamani, 2019 ; Sharma *et al.*, 2019 ; Shnoudeh *et al.*, 2019 ; Parupudi *et al.*, 2022)**. Therefore, the measurement of this parameter provides an understanding of the dispersion, aggregation or flocculation of the particles in a solution and by that their mobility and conductivity.

The measurement of zeta-potential and mobility and of the digested samples was carried using the Malvern Zetasizer Nano (Malvern, UK). 15 scans were performed for each sample after being inserted to the Zetasizer cuvette at a persistent voltage of 40mV and temperature of

25°C. The DTS software of the Malvern instrument was used for calculating the three parameters. All experiments were performed in triplicate **(Zadaka *et al.*, 2010 ; Clogston *et* Patri, 2011)**.

II.4. Therapeutical activities of the samples' hydrolysates

Due to their huge benefits to the human health, neuroprotective, anti-diabetic, anti-obesity and antioxidant capacities of the hydrolysates' samples were tested.

In all applied assays the IC50 known as the inhibitory concentration at 50% was determined for all the samples. The IC50 values of all the samples (n=3) was calculated according to the drawing graphs obtained from the inhibition ratio of each concentration in which the equation is mentioned at the end of each assay protocol). The resulted curve equation is then applying for the IC50 calculation (by replacing the Y by 50), as demonstrated in the following figure:

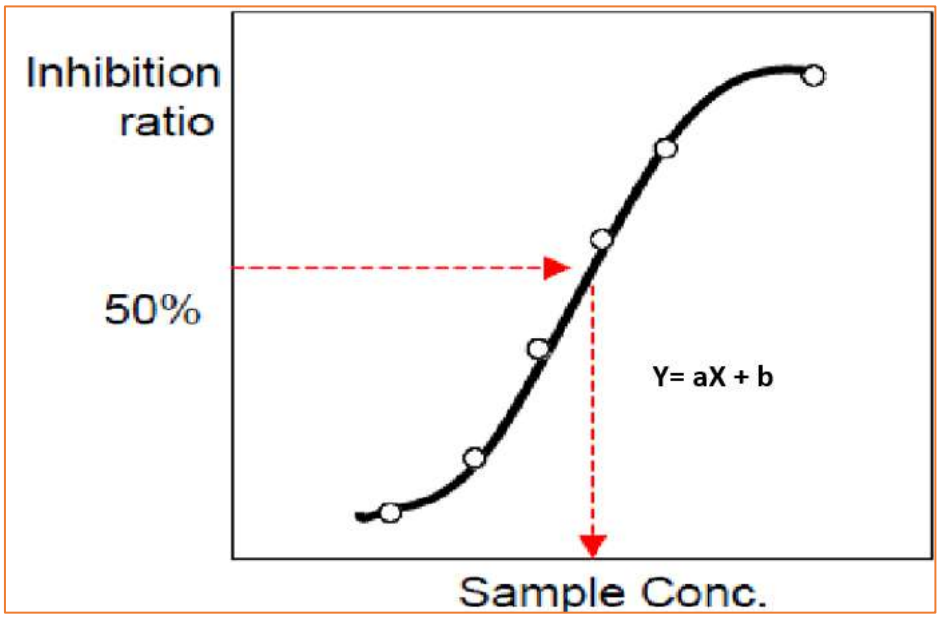

Figure 9. Inhibition ratio curve of sample concentrations

II.4.1. Neuroprotective activity

Neuro-damaging can be caused by several factors and results multiple neural disorders. As mentioned earlier, one of the most popular diseases that can be produced by this damaging is the Alzheimer, which is caused by the high activity of the AChE enzyme **(Ferreira-Vieira *et al.*, 2016 ; Breijyeh *et* Karaman, 2020 ; Marucci *et al.*, 2021)**. Therefore, we aimed to investigate one of the undetermined capacities of the DM, DU and their mixture by evaluating their inhibition capacity against the AChE enzyme.

II.4.1.1. Inhibition of the acetylcholinesterase

Acetylcholinesterase (AChE) (EC 3.1.1.7) is an enzyme involved in the transmission of nerve impulses. Belongs to the cholinesterase's family, found mostly in nerve tissue and red blood cells **(Colović *et al.*, 2013 ; Marucci *et al.*, 2021)**. To inhibit the transmission of nerve signals through the synapse at neuromuscular junction, the AChE function is to hydrolyze the neurotransmitter acetylcholine into acetic acid and choline in synaptic clefts **(Colovic *et al.*, 2013 ; Trang *et* Khandhar, 2021)**.

AChE exists in several molecular forms which are monomer, polymer or asymmetric form provided with a collagen tail. It acts mainly in the central nervous system (CNS), on neuromuscular synapses **(Xu *et al.*, 2017 ; Trang *et* Khandhar, 2021)**. This enzyme is also known by its fast activity with a turnover of an average 10000 molecules of substrate hydrolyzed per second (between 1000 and 20000 s^{-1}, depending on the species type). The high speed is necessary to keep the excitations transmitted time interval by the neurons as short as possible **(Colovic *et al.*, 2013 ; Trang *et* Khandhar, 2021)**.

II.4.1.2. Assay principal

Various assays and methods for determination of the AChE inhibitory activity of bio-active compounds have been reported. However, the colorimetric procedure of **Ellman *et al.* (1961)** was the chosen method in this study. The Acetylthiocholine (ATCh) was used as the substrate of this enzyme instead of acetylcholine (ACh). The hydrolysis of the ATCh by the AChE releases the thiocholine that reacts with DTNB, producing TNB (5-thio-2-nitrobenzoate), a compound with a yellow color which can be detected by spectrophotometry at 412 nm (Figure 9). The absorbance is proportional to the enzymatic activity of the AChE. The more the enzyme is blocked, the less the yellow compound is produced **(Arduini *et al.*, 2007 ; Pohanka *et al.*, 2011)**.

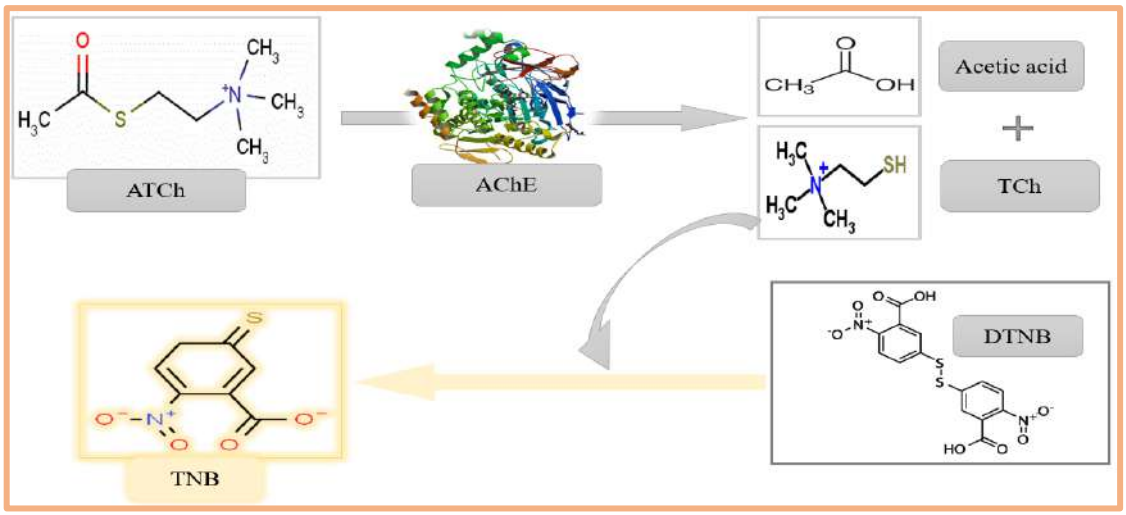

Figure 10. Measurement principle of the AChE activity by spectrophotometric method.

II.4.1.3. Inhibition assay of AChE activity

The colorimetric procedure was used to evaluate the activity of acetylcholinesterase by carrying a slight modification on the method described by Elman *et al.,* (1961). In each hall of a microplate, 50µl of Tris-HCl buffer (pH 8), different concentrations of the hydrolysates and 0.5U/ml of enzyme were introduced. The mixture was then incubated for 15 min in a dark place with continuous agitation at room temperature. After that, 75µl of acetylthiocholine iodide (AChI) (1.5 mM) and 125µl of DTNB (3mM) were added. The galanthamine hydrobromide was the positive control in this assay **(Ingkaninan *et al.*, 2003 ; Pohanka *et al.*, 2011 ; Heo *et al.*, 2020)**.

The AChE inhibition activity was calculated after measuring the mixtures absorbance at 412 nm according to the following formula (Formula 1) :

$$I\% = (Ab - ((As - Abck)/ab))) * 100$$

Where As is the sample absorbance, Ab is he blank absorbance and Abck is the back ground absorbance.

II.4.2. Antioxidant acitivity

Antioxidant activity has become one of the most studied activities of the natural substances due to its major importance. Most of health disorders that can touch the human body are caused by free radicals and ions activity **(Pham-Huy *et al.*, 2008 ; Maddu, 2019)**. So far, several tests are applied to determine the antioxidant capacity of any compound.

The antioxidant activity of the DMH, DUH and the two formula hydrolysates were determined applying two different methods which were radical scavenging using the free radical ABTS and reducing power ability using the FRAP test. The ascorbic acid was used as a control and the IC_{50} values (otherwise known as the inhibitory concentration at 50%) were determined using the concentrations of the solid materials in all hydrolysates'samples **(Kayacan *et al.*, 2020).**

II.4.2.1. ABTS radical scavenging

In order to determine the scavenging ability of a compound against the free ABTS radical, it's necessary to oxidize the ABTS molecule with the potassium persulfate to produce the $ABTS^{+}$ **(Dong *et al.*, 2015 ; Otieno *et al.*, 2016)**. This cationic radical is characterized with a bluish-green color. The trapping of this radical cause a disappearance of its color. So the antioxidant activity in this case is proportional to the decreasing of the bleu-green color detected spectrophotometrically at a wavelength of 734 nm **(Dasgupta *et* Klein, 2014 ; Dong *et al.*, 2015 ; Otieno *et al.*, 2016)**. The principal of this assay is illustrated in the figure 10.

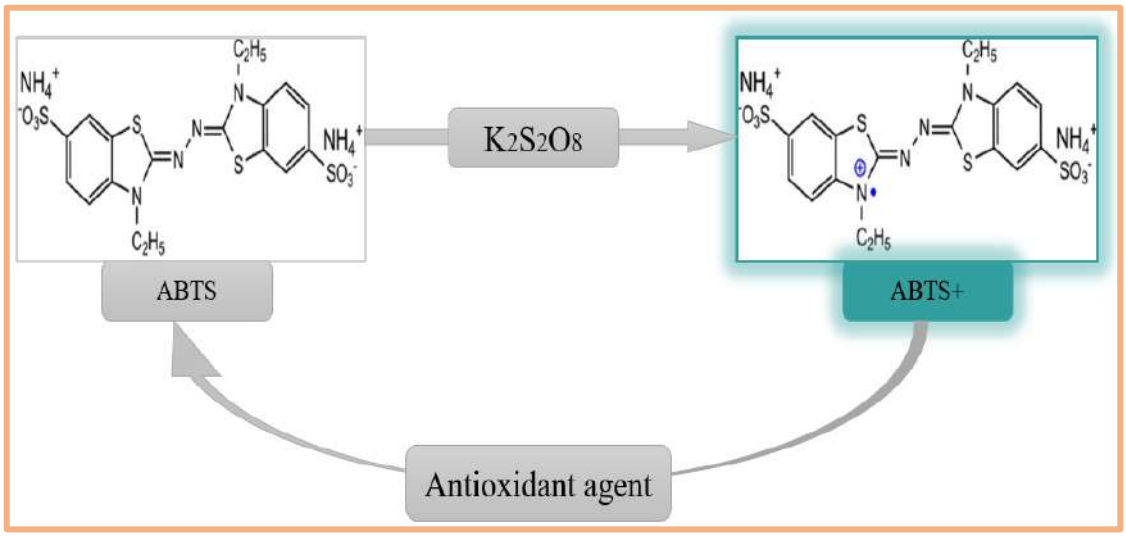

Figure 11. Reaction schema of the ABTS radical scavenging

II.4.2.1.1. ABTS scavenging assay

Determination of ABTS radical inhibition process was performed according to the method described by **Re *et al.*, (1999)**. A 0.05ml of each hydrolysate sample with PB at pH 7.4 as blank was added to 1.95ml of a diluted ABTS solution prepared 16h earlier. The free radical solution was prepared by mixing 10ml of a methanolic ABTS solution (14 mM) and 10 ml of a

4.9 mM methanolic ammonium persulfate solution with an absorbance of 0.700 ± 0.02 at 734 nm. Afterwards, the reactional tubes were incubated in a dark place for 6-7 min, so finally the absorbance was measured at 734 nm. The following equation was applied to calculate the percentage of the inhibition ratio (IR) **(Gómez-Ruiz *et al.*, 2007 ; Suseela *et al.*, 2010)**.

IR= ((Ab of the blank-Ab of the sample)/ Ab of the blank)*100

II.4.2.2. Ferric reducing antioxidant power (FRAP)

The FRAP method is based on the reduction of ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}). This method evaluates the reducing power of compounds **(Ou *et al.*, 2001)**. The presence of reducing agents causes the reduction of Fe^{3+}-ferricyanide complex to the ferrous form. Therefore, Fe^{2+} can be evaluated by measuring and monitoring the increase of the navy-blue color density of this compound in the reaction medium at 700 nm (Figure 11) **(Chung *et al.*, 2002)**.

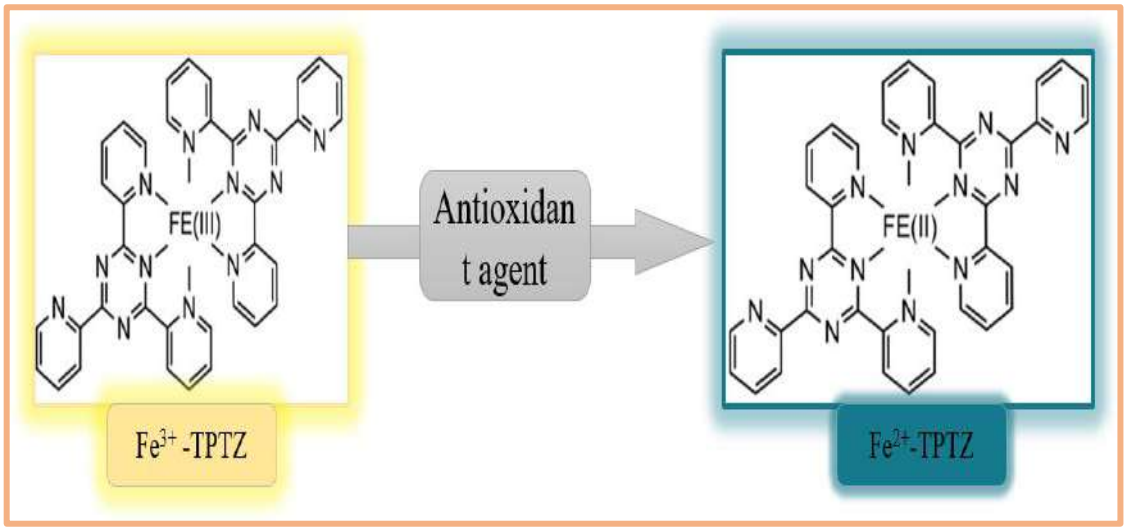

Figure 12. Reaction schema of reduction by antioxidant agent of the ferric ion (Fe^{3+}) into ferrous ion (Fe^{2+})

II.4.2.2.1. The FRAP assay

The method described by **Oyaizu, (1986)** was followed to evaluate the reducing power of iron (Fe^{3+}) of the hydrolysate samples. One milliliter of each sample at different concentrations was mixed with 2.5 ml of phosphate buffer solution (0.2 M, pH 6.6) and 2.5 ml of $K_3Fe(CN)_6$ solution (1% (w: v)). The mixture was incubated at 50 °C for 20 min. Afterwards,

1 ml of a buffer containing 10% of trichloroacetic acid (TCA) was added to stop the reaction. The tubes were further centrifuged at 3000 rpm for 10 min. 2.5 ml of the supernatant was combined with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ solution. Eventually, the absorbance of the prepared mixture was measured at 700 nm.

The increase in absorbance corresponds to an increase in the reducing power of the tested samples. The IC_{50} corresponds to the 0.5 of the absorbance calculated using the acquired by insertion of the linear regression analysis **(Goudjil *et al.*, 2020)**.

II.4.3. *In vitro* antidiabetic activity

The antidiabetic ability of the hydrolysates samples was evaluated *in vitro* by investigating the inhibition capacity of the hydrolysates against α-amylase and α-glucosidase. The two key enzymes are involved in the digestion process of polysaccharides into monosaccharides that moves to the internal body in the blood circulation.

II.4.3.1. α-amylase inhibition capacity

The α-amylase or the 1,4-alpha-D-glucan glucanohydrolase (EC 3.2.1.1) is an endoenzyme with a molecular mass between 40 and 70 kDa. It is considered as glycoprotein containing 478 amino acids divided into 3 globular domains **(Igarashi *et al.*, 1998 ; Robert *et al.*, 2003 ; Nouadri, 2011)**. These domains are associated by a polypeptide chain consisting mainly of hydrophobic residues. The residues constituting the substrate binding site as well as the catalytic site are located in the A domain which shows that α-amylase is formed of 8 folded β sheets and 8 α helices **(Ohdan *et al.*, 1999 ; Franco *et al.*, 2002 ; Robert *et al.*, 2003 ; Dong *et al.*, 2019)**.

The α-amylase is a digestive enzyme that hydrolyses polysaccharides, secreted mainly by the salivary glands and the pancreas. It required for the catabolism of long chain carbohydrates into smaller units. It hydrolyzes the α-D-glucose polymers at α (1-4) linkages of amylose, amylopectin, starch and glycogen excluding the terminal bonds of these chains resulting the release of glucose, maltose and especially α-dextrin **(NieuwAmerongen *et al.*, 1981 ; Freers, 2005 ; Mikawlrawng, 2016)**.

II.4.3.1.1. Assay principal

Since the role of α-amylase enzyme is mainly related to the digestion of the starch, the chosen protocol in this study meant the use of the starch as a substrate for the α-amylase that causes the release of glucose and maltose. Those monosaccharaides are confederated as a reducing sugar that can act with different coulometric agents such as the 3,5-dinitro salicylic

acid (DNS) which is characterized by an intense yellow color (Figure 12). In alkaline condition, at the presence of a reducing sugar and at a high temperature (100°C), the DNS changes to 3-amino, 5-nitrosalicylic acid (ANSA) with an orange-red color easily measured spectrophotometrically at 540 nm **(Miller, 1959 ; Wood *et al.*, 2012 Deshavath *et al.*, 2020)** (Figure 12). Following this principal, the determination of DMH, DUH, DMUH1and DMUH2 inhibition capacity was investigated.

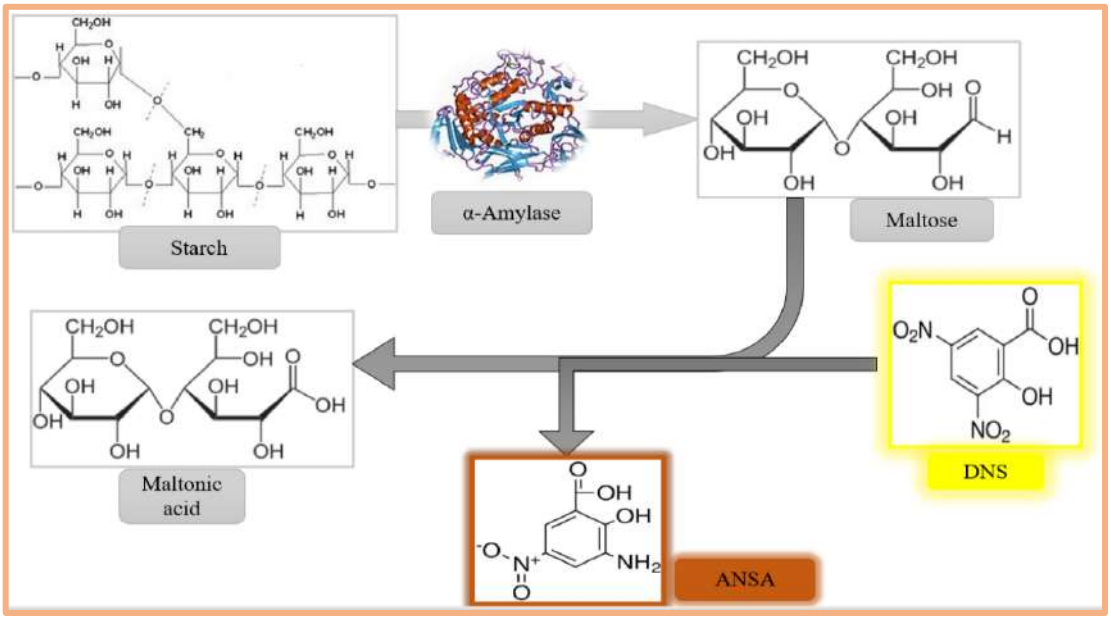

Figure 13. Measurement principle of the α-amylase activity by spectrophotometric method.

II.4.3.1.2. Inhibition assay of α-amylase activity

The effect of the digested samples on the catalytic activity of α-amylase was carried out according to **Lalegani *et al.* (2018)** method with a slight modification. To 80µl of varying concentrations of SH was added 500µl of 0.02 M sodium phosphate buffer (pH 6.7) with α-amylase solution (500µg/ml). After incubation for ten minutes in a dark place, 100µl of starch solution (1%) was introduced to the reaction mixture. The reaction tubes were left for ten minutes at 37°C in the dark with continuous agitation. A volume of 200µl of DNS (the colour indicator) was added to the previous solution and heated at 100°C to stop the reaction. The colour of the reaction was detected at 540nm **(Lalegani *et al.*, 2018)**. The positive control in this case was the acarbose. The inhibition capacity of the digested samples against the α-amylase was determined by applying the formula 1.

$$I\% = ((Ab - ((As - Abck))/Ab) * 100$$

II.4.3.2. α-glucosidase inhibition

α-glucosidases (EC 3.2.1.20), enzyme hydrolyzing polysaccharides, oligosaccharides and di-saccharides into monosaccharides was selected to determine the *in vitro* antidiabetic ability of the hydrolysates' samples.

The Carbohydrate-hydrolase act on hydrolyzing mainly the α-1,4-glucosidic bonds of a substrate either at the terminal or in the non-reducing terminal position. For example, the malto-oligosaccharides or glycogen digestion releases the α-D-glucose. They can also catalyze trans-glycosylation reactions to synthesize α-D-glycosylated compounds **(Chinnery *et* Turnbull, 2007 ; Abbas *et al.*, 2017)**.

II.4.3.2.1. Assay principal

Based on its cutting position, the activity of this enzyme can be measured by the using the colorless molecule of the p-nitrophenyl-α-d-glucopyranoside (pNPG), a chromogenic substrate that contains a glycosidic bound between the D-glucose and the p-nitrophenyl. Breaking this bound by the α-glucosidase releases the p-nitrophenyl, which is characterized by a yellow color detected spectrophotometrically at 405nm **(Elya *et al.*, 2012 ; Mohiuddin *et al.*, 2016 ; Promyos *et al.*, 2017)**.

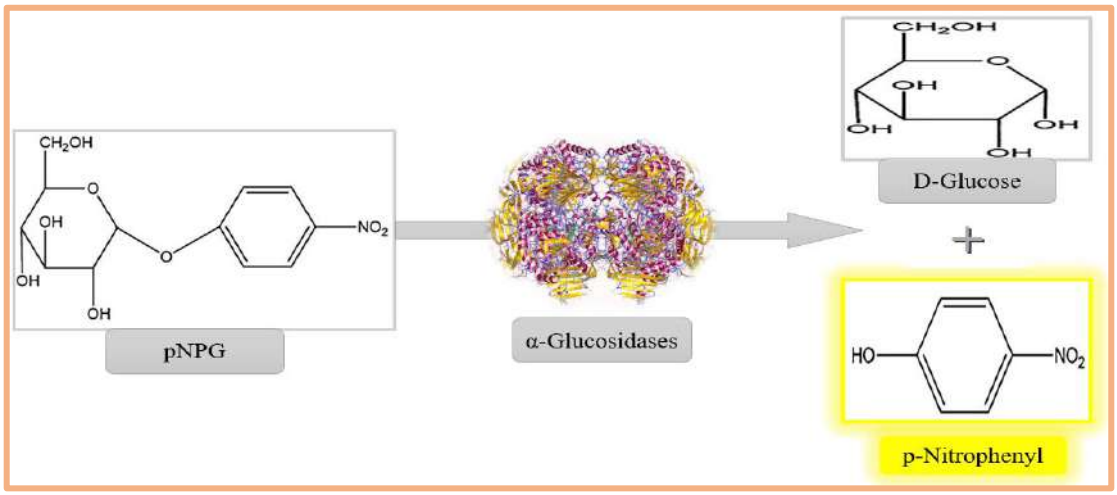

Figure 14. Measurement principle of the α-glucosidase activity by spectrophotometric method.

II.4.3.2.1. Inhibition assay of α-glucosidase activity

The ability of the four hydrolysates samples to inhibit the α-glucosidase activity was determined as reported by **Lalegani *et al.*, (2018)**, with some changes. The reaction mixture consisting of 1ml of phosphate buffer (50 mM, pH 6.8), 50 µl of α-glucosidase (4 U/ml) and 50 µl of hydrolysates varying concentrations was pre-incubated at 37°C for 10 min. Then, 100µl of p-NPG (10 mM) was added as substrate and reincubated at 37°C for 30 min. The reaction

was stopped by adding 1ml of sodium carbonate (0.2mM). The yellow colour produced was read at 405 nm **(Lalegani *et al.*, 2018)**. Acarbose at various concentrations (0.2 - 1 mg/ml) was token as a positive control. Applying formula 1, the calculation of the sample's inhibition capacity was carried out.

II.4.4. Pancreatic lipase inhibition capacity

Lipase, also called triacylglycerol acyl hydrolases (EC.3.1.1.3) is a glycoprotein mainly secreted by the pancreas. It is present in small quantities in the gastric mucosa. It is released by the digestive tract for the digestion of fats which can be absorbed by the small intestine and used by the body to provide it with energy **(Miled *et al.*, 2000 ; Bauer *et al.*, 2005)**.

Lipases belong to the family of carboxylic ester hydrolases. Its physiological role is to hydrolyze triglycerides into diglycerides, monoglycerides, fatty acids and glycerol. Hydrolysis of ester bonds of water-insoluble lipid substrates occurs at the interface between lipid and water. These enzymes, present in all living organisms, play a key role in the biochemistry of lipids **(Hou *et* Shimada, 2009 ; Brockman, 2013)**. Lipases also have the ability to carry out synthetic reactions such as esterification (reaction between acid and alcohol), transesterification (ester and alcohol) and interesterification (ester and ester) as well as in transfer reactions of the acetyl group of an ester on other nucleophiles such as amines or thiols **(Alloue *et al.*, 2008 ; Zieniuk *et al.*, 2021 ; Reyes-Reyes *et al.*, 2022)**.

II.4.4.1. Assay principal

Most of the used assay to evaluate the P-lipase activity are founded on its hydrolysis point which is the ester link holding a fatty acid to a glycerol molecule. Therefore, breaking the bound between a fatty acid and a compound that become colorful after hydrolysis, allowing the estimation of the enzyme activity. Several substrates are used for this reason that are count to be a fatty acid linked to the p-nitrophenyl with an ester bound. The lipase activity is measured by the yellow colour intensity of the liberated p-nitrophenyl **(Stoytcheva *et al.*, 2012 ; Hu *et al.*, 2015 ; Santana *et al.*, 2017)**.

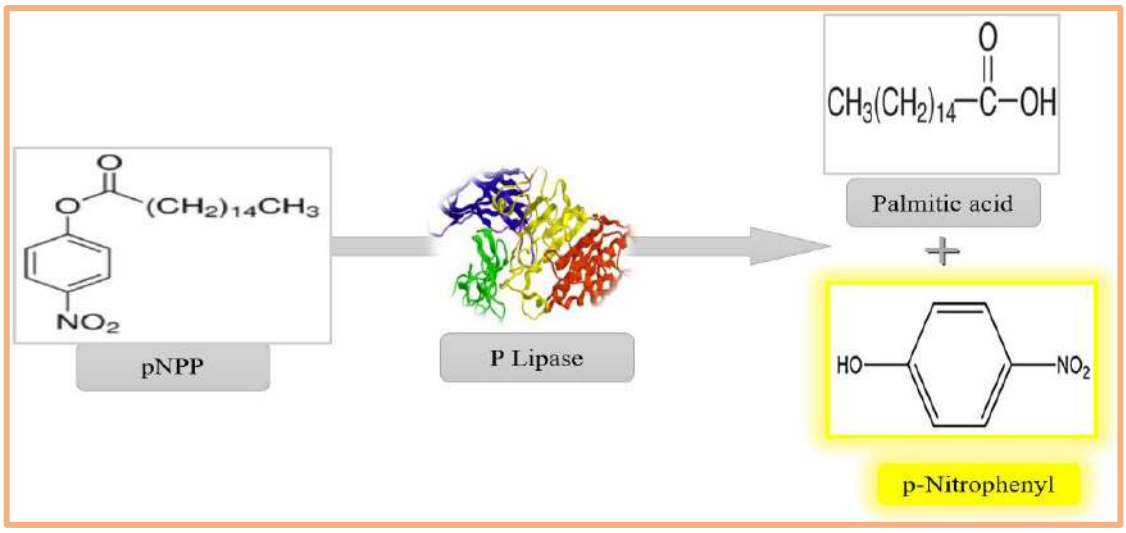

Figure 15. Measurement principle of the pancreatic lipase activity by spectrophotometric method.

II.4.4.2. Inhibition assay of Pancreatic lipase activity

The digested samples' ability to inhibit the P-lipase activity was carried out after effecting a slight modification on the method described by Qui and Zhang (2019); using the palmitate-p-nitrophenyl as substrate.

In 0.9 ml of Tris-HCl buffer (pH 8.5), containing SDS and 2% of Trion-X,100 µl of the varying concentration of the digested samples was incubated with 100 µl of p-nitrophenyl (PNPP) substrate for 5 min at 37°C. After that, the reaction was commenced with the addition of the enzymatic solution and the mixture was incubated for 30 min at 37°C in the dark. The released p-nitrophenol was detected at 410 nm **(Qiu *et* Zhang, 2019)**. The positive control was the Orlistat. The calculation of the inhibition ability of the samples was determined using formula (1).

II.5. Statistical analysis

The statistical analyses of this study were exhibits with the use of two tools. The S.P.S.S. package program (IMB SPSS 25) applying the One-way analysis of variance method (ANOVA). The variance between the means of each test of each samples' concentrations was performed in triplicate (n=3) and assessed using the Tukey test at a significance level of 0.05. The Principal Component Analysis (PCA) was carried out with the Excel software (XLSTAT 2016.02.28451).

Results & discussion

III. Results and discussion

III.1. Samples characterization

III.1.1. Physical and chemical characterization

III.1.1.1. pH

The Hydrogen potential values of the dromedary milk, dromedary urine and their mixtures are presented in table IV.

		DM	DMU1	DMU2	DU
pH	**Min**	6,003±0,006$^{(d)}$	6,337±0,006$^{(b)}$	6,293±0,006$^{(c)}$	7,120±0,000$^{(a)}$
	Max	6,537±0,040$^{(d)}$	7,200±0,000$^{(c)}$	7,547±0,006$^{(b)}$	8,393±0,006$^{(a)}$
	Mean	6,269±0,059$^{(d)}$	6,898±0,006$^{(c)}$	7,153±0,001$^{(b)}$	8,133±0,003$^{(a)}$

Table IV. pH values of dromedary milk, urine and their mixtures.

$^{a-d}$: Different letters in the line indicate a significant difference (P <0.05) between the deferent samples.

The lowest level pH mean of 6.269±0.059 was documented by the DM samples, while the highest pH average of 8.133±0.003was recorded by the urine samples. Moreover, the milk samples pH varies between 6.003±0.006 and 6.537±0.040 and the pH rate of the urine samples goes from 7,120±0,000 to 8,393±0,006. Despite the age, the feeding and the lactation stage dissimilarity between the camels, DM samples demonstrated no statistically significant difference between all the tested samples ($p<0.05$) (Table IV).

The average of all dromedary milk (DM) samples within the general range of the dromedary milk pH that goes from 5.7 to 6.7 as reported in several works **(Bouhaddaoui *et al.*, 2019 ; Swelum *et al.*, 2021)**. Nevertheless, the obtained DM pH mean also aligned with the average pH documented from multiple studies on the Algerian dromedary milk that varies between 4.9 and 6.86 **(Henni *et* Kheroua, 2005 ; Hadef *et al.*, 2018 ; Zahra *et al.*, 2021)**.

The slightly acid pH of DM can be due to its important vitamin C content that can exceed 24.9mg/l (**Bouhaddaoui *et al.*, 2019)**. Moreover, in addition to the food and water consumed by the animal, caseins and citrate concentration can also influence the pH level of this milk **(Broyard *et* Gaucheron, 2015 ; Schiffer *et al.*, 2021)**

Contrary to DM, the urine (DU) exhibited a bit basic pH level as reported by previous studies **(Al-Yousef *et al.*, 2012 ; Khedr *et* Khorshid, 2017 ; Salamt *et al.*, 2021)**. The slight

notable pH difference between the urine samples is mostly due to the age and food intake dissimilarity **(Salamt *et al.*, 2021)**.

The two formulas of milk-urine showed an augmentation in the pH level comparing to the DM samples, yet lower than the pH demonstrated by the urine samples. The DMU1 illustrated a mean pH value of 6.898±0.006 with an interval that starts with 6.337±0.006 and ends at 7.200±0.000. In the other hand, the DMU2 pH mean value was 7.153±0.001 with 6.293±0.006 and 7.547±0.006 as the minimum and the maximum values, respectively (Table IV).

The neutral pH of DMU1 and DMU2 can be due to the urine addition. Changing the pH level counts as modifying the general proprieties of the milk which can affect its constituent in general and proteins in specific causing a modification in the atoms' protonation state of amino acids, **(Bischoff *et* Schlüter, 2012 ; Di Russo *et al.*, 2012 ; Omar *et al.*, 2016 ; Singh, 2011)**. These modifications can influence their general functionality and solubility which also can negatively affect the digestion process of those proteins **(Di Russo *et al.*, 2012 ; Talley *et* Alexov, 2010)**.

The increasing pH of both caused by the addition of the DU can affect the DM's proteins mobility, conformation and hydrolysis.

III.1.1.2. Total soluble solids contents

The total soluble solids amount (Brix), expressed in percentage of the four tested samples DM, DU, DMU1 and DMU2 is illustrated in table V.

		DM	DMU1	DMU2	DU
Brix %	**Min**	6.133±0.115$^{(a)}$	5.767±0.058$^{(b)}$	5.333±0.115$^{(c)}$	4.133±0.115$^{(d)}$
	Max	8.000±0.000$^{(d)}$	8.200±0.000$^{(c)}$	8.867±0.115$^{(b))}$	12.000±0.000$^{(a)}$
	Mean	6.892±0.014$^{(b)}$	7.058±0.556$^{(b)}$	6.654±0.044$^{(b)}$	8.092±0/014$^{(a)}$

Table V. Brix of dromedary milk, urine and their mixtures.

$^{a-d}$: Different letters in the line indicate a significant difference (P <0.05) between the results of different camel samples. DM: Dromedary milk, DMUA: Dromedary Milk-Urine mixture 9-1 (v/v), DMU2: Dromedary Milk-Urine mixture 8-2 (v/v), DU: Dromedary urine.

A large variation in the TSS level was registrated especially by the DU samples which recorded a variation that goes from 4.133±0.115 to 12.00±0.00. Furthermore, the greater Brix level mean was expressed by the urine samples. The DM, DMU1 and DMU2 samples

recorded a close Brix level yet with a significant difference, where the TSS amount was 6.892±0.014, 7.058±0.556 and 6.654±0.044, respectively. Moreover, no remarkable variance was found between the TSS maximum values of the three milk samples ($p<0.05$) (Table V).

The documented amount of the DM's TSS is count to be significantly low comparing to the global average of camel milk TSS registrated in multiple works **(Alhaj *et al.*, 2022 ; Habte *et al.*, 2018 ; Zibaee *et al.*, 2015)**. Noting that this parameter is directly associated to the season that rules the feeding system in general and the water consummation in specific by this animal **(Singh *et al.*, 2017)**. In fact, the more water is consumed by the dromedary the less soluble solids are in the milk **(Singh *et al.*, 2017)**.

The investigated parameter in the urine samples is also related to the water consumption **(Ali *et al.*, 2019)**. Indeed, the dromedary's kidneys characterized by an important ability to reabsorb water as a conservation path resulting the secretion of high level of soluble solids **(Abdalla, 2020 ; Kataria *et al.*, 2007 ; Ali *et al.*, 2019)**.

The difference observed in the brix level can be due to the distinction between the animals in age, lactation stage and food and water intake **(Kataria *et al.*, 2007 ; Haddadin *et al.*, 2008 ; Singh *et al.*, 2017 ; Ali *et al.*, 2019).**

The decrease in the TSS values of both mixtures indicates the existence of a molecular interaction between both liquids' constituents **(Tran, 2020)**.

The two mixtures recorded a TSS content lower than the two liquids alone, highlighting the formation of new bounds between the DM and DU's compounds.

III.1.1.3. Optical proprieties

DM, DU, DMU1 and DMU2 optical proprieties and color characteristics are presented in table VI.

Table VI. Optical proprieties of the dromedary milk, urine and their mixtures.

		DM	DMU1	DMU2	DU
a*	**Min**	-1.690±0.017$^{(a)}$	-2.253±0.025$^{(b)}$	-3.090±0.046$^{(d)}$	-2.640±0.017$^{(c)}$
	Max	-0.893±0.015$^{(a)}$	-1.277±0.081$^{(b)}$	-1.840±0.020$^{(c)}$	-0.887±0.212$^{(a)}$
	Mean	-1.245±0.014$^{(a)}$	-1.765±0.025$^{(b)}$	-2.417±0.013$^{(c)}$	-1.713±0.049$^{(b)}$
b*	**Min**	1.060±0.053$^{(d)}$	1.343±0.121$^{(c)}$	1.747±0.168$^{(b)}$	5.393±0.035$^{(a)}$
	Max	4.867±0.040$^{(d)}$	6.163±0.040$^{(c)}$	12.793±0.898$^{(a)}$	10.827±0.158$^{(b)}$
	Mean	2.491±0.078$^{(c)}$	3.305±0.328$^{(c)}$	5.689±0.541$^{(b)}$	7.769±0.379$^{(a)}$
	Min	81.993±0,690$^{(a)}$	76.340±0.567$^{(b)}$	74.143±0.628$^{(c)}$	14.723±0.031$^{(d)}$

L*	**Max**	90.597±0.152[(a)]	85.223±0.207[(c)]	87.483±0.229[(b)]	20.830±0.436[(d)]
	Mean	86.970±0.107[(a)]	80.852±1.241[(b)]	79.818±1.274[(b)]	17.233±0.164[(c)]

[a-d]: Different letters in the line indicate a significant difference (P <0.05) between the results of different camel samples. DM: Dromedary milk, DMUA: Dromedary Milk-Urine mixture 9-1 (v/v), DMU2: Dromedary Milk-Urine mixture 8-2 (v/v), DU: Dromedary urine.

As table VI represents, all the DM samples showed high *L** parameter values with a mean of 86.970±0.107, five time higher than the average recorded by the urine that showed an average of 17.233±0.164.

The DMU1 and DMU2 showed a decrease in the *L** indicator comparing to the milk sample. In fact, the more urine is added the lower *L** parameter gets with an average of 80.852±1.241 and 79.818±1.274 for the two mixtures, respectively (Table VI).

The very bright color of the DM can be related to the important calcium content and to the protein's concentration in this milk, in addition to the perfect homogenization and distribution between the fats and casein micelles in the hall milk **(Bakry *et al.*, 2021)**.

In opposite the dark character or the optical proprieties in general of the DU was never being documented previously. However, it may be caused by the water re-absorbance by the dromedary kidneys resulting the production of an incredibly concentrated urine **(Abdalla, 2020)** leading to its dusky and intense color.

The four tested samples registered negatives *a** character values. The highest mean for this parameter was -1.245±0.014, recorded by the DM, followed by the DMU1 with an average of -1.765±0.025 and the DMU2 with a mean of -2.417±0.013. The lowest mean value for the *a** character of -1.713±0.049 was registrated by the urine samples (Table VI).

For the *b** parameter, positive values were recorded by all the samples. The urine samples revealed the highest mean value of 7.769±0.379, a value that gradually decrease with decreasing of urine concentration in both formulas to reach the lowest values in the DM sample. Where, DMU2, DMU1 and DM demonstrated an average of 5.689±0.541 and 3.305±0.328 and 2.491±0.078, respectively (Table VI). In which, the mean values represented by the DM samples count to be the lowest performance for the *b** indicator.

The documented results found to be in a total accordance with the eyes visualization of the DM, DU and the two mixtures. In spite of that, these results are not in comparable position due to the major lack of previous data. Nevertheless, both mixtures appeared to be very approximate to the milk samples consequently to low dose of the incorporated urine and the good homogenization and integration of its molecule with the ones of milk.

III.1.2. Biochemical characterization

III.1.2.1. Sugar, proteins and fat contents

Sugar, proteins and fat mean contents in the DM, DU, DMU1 and DMU2 are illustrated in figure 15. Those compounds minimal and maximal concentrations are shown in table VII.

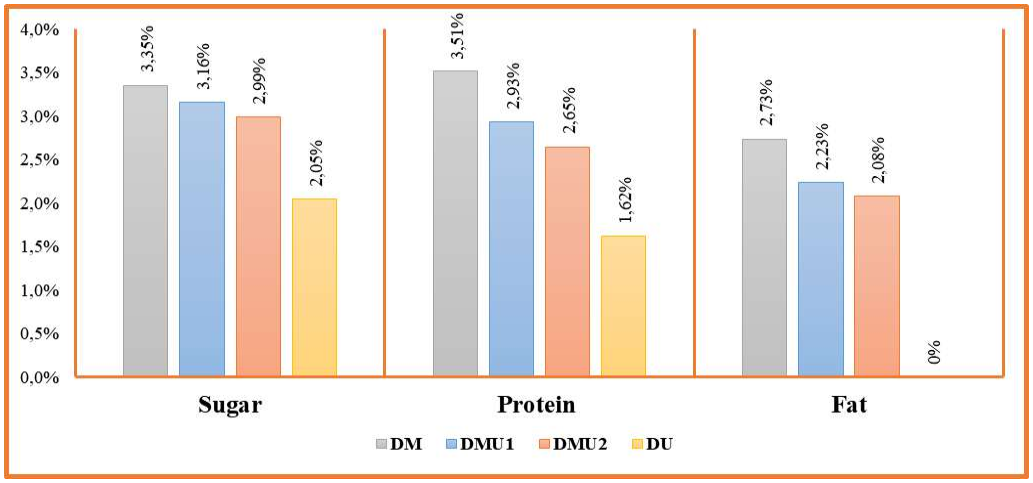

Figure 15. Sugar, proteins and fat contents percentage in dromedary milk, dromedary urine, and their mixtures

DM samples showed a content of 3.353±0.405%, 3.513±0.185% and 2.733±0.145% in sugar, protein and fat, respectively. Compared to the DM, a remarkable decrease in DMU1 and DMU2 concentrations of those compounds was registrated, where the DMU1 recorded 3.155±0.376% of sugar, 2.928±0.0% of proteins and 272.233±0.051% of fats. While, the DMU2 recorded an amount of 2.987±0.474%, 2.645±0.026% and 2.075±0,058% for the sugar, proteins and fat, respectively (Figure 15).

The urine sample showed molecules containing the Amid I bound of 1.617±0.034 % revelated by the FTIR technique and a sugar percentage of 2.046±0.456% (Figure15), (TableVII).

Table VII. Biochemical parameters of the tested samples.

		DM	DMU1	DMU2	DU
Sugar	**Min**	2.707±0.090$^{(a)}$	2.658±0.030$^{(a)}$	2.301±0.080$^{(b)}$	1.271±0.033$^{(c)}$
	Max	3.741±0.021$^{(b)}$	3.681±0.064$^{(b)}$	3.898±0.046$^{(a)}$	2.641±0.064$^{(c)}$
	Mean	3.353±0.405$^{(a)}$	3.155±0.376$^{(b)}$	2.987±0.474$^{(b)}$	2.046±0.456$^{(c)}$
Protein	**Min**	2.441±0.0116$^{(a)}$	2.236±0.054$^{(b)}$	2.073±0.049$^{(b)}$	1.243±0.022$^{(c)}$
	Max	3.895±0.025$^{(a)}$	3.669±0.075$^{(b)}$	3.304±0.075$^{(c)}$	2.274±0.059$^{(d)}$
	Mean	3.513±0.185$^{(a)}$	2.928±0.027$^{(b)}$	2.645±0.026$^{(c)}$	1.617±0.034$^{(d)}$
Fat	**Min**	1.833±0.058$^{(a)}$	1.467±0.058$^{(b)}$	1.133±0.057$^{(c)}$	/
	Max	3.500±0.000$^{(a)}$	3.300±0.000$^{(b)}$	3.067±0.058$^{(c)}$	/
	Mean	2.733±0.145$^{(a)}$	2.233±0.051$^{(b)}$	2.075±0,058$^{(b)}$	/

$^{a-d}$: Different letters in the line indicate a significant difference (P <0.05) between the results of different camel samples. DM: Dromedary milk, DMUA: Dromedary Milk-Urine mixture 9-1 (v/v), DMU2: Dromedary Milk-Urine mixture 8-2 (v/v), DU: Dromedary urine.

The acquired proteins amount existing in the milk samples using the FTIR technique was incredibly compatible with the main average of proteins content in dromedary **(Urbisinov Zh *et al.*, 1981 ; Zibaee *et al.*, 2015 ; Konuspayeva *et* Faye, 2021; Swelum *et al.*, 2021)**. Nevertheless, the founded results agrees with the ones declared by several works carried on the Algerian dromedary milk using the spectrophotometric methods such as Bradford and Biuret, additionally to the Kjeldahl method which based on the total nitrogen calculation **(Hadef *et al.*, 2018 ; Zahra *et al.*, 2021)**.

Moreover, the reached concentrations integrating the FTIR method agrees with the content reported by **Mohamed *et al.*, (2021)** applying near and mid infra-red spectroscopy. However, the slight remarkable difference in the protein content can be due to the no-similarity of the lactation stage and the age **(Fekadu *et al.*, 2005 ; Singh *et al.*, 2017 ; Akindykova *et al.*, 2019)**. In addition, the sampling was carried out in the period where the desert reaches its poverty in the plant species leading to an extremely lack in the food source, affecting by that the concentration of the organic compounds in milk such as proteins **(Yang *et al.*, 2013 ; Sisay *et* Awoke, 2015 ; Oselu *et al.*, 2022, , Vincenzetti *et al.*, 2022)**.

The obtained results by the FTIR of the urines samples don't reflect the number of proteins, but it represents the concentration of the compounds containing the amide I group such as peptides with molecular wight beneath the 10KDa in addition to nitrogenous molecule as urea, uric-acid and creatinine that contains **(Sarigul *et al.*, 2021)**. However, those compounds seem to be present in a low quantity in a comparison with the TSS amount. Where this animal kidneys show the capacity to reuse the nitrogen of those molecules in other

biochemical pathways like protein production. Therefore, the dromedary urine is count to be safe to the consumers due the low concentration of those toxic compound **(Ahamad *et al.*, 2017)**.

The protein content in both mixtures was not proportional to the concentration of the milk after eliminating the 10% and 20% and adding the urine instead. The decreasing in the molecule containing the amid I concentration could be caused by the creation of new bounds involves the C=O group including the milk proteins ones. Interaction that can influence milk proteins proprieties and capacities, especially that most of the value of the DM is linked directly proteins **(Choudhary *et al.*, 2009 ; Bartlett *et al.*, 2010 ; Al-Juboori *et al.*, 2013 ; Baronio *et* Barth, 2020)**.

The two mixtures show the possible existence of new amidic bound between the two liquids after being combined which may affect the milk protein capacities and functionality.

The sugar DM content present mostly the lactose concentration which run in the same flow with the percentages reported by precedent works, despite the utilization of FTIR **(Yoganandi *et al.*, 2015 ; Zibaee *et al.*, 2015 ; Hammam *et* Agriculture, 2019 ; Ho *et al.*, 2021)**.

The bit difference of the lactose content comparing to one declared by **Mohamed *et al.*, (2021)**, where the near and mid infra-red spectroscopy was applied may be related to the food intake **(Al-Juboori *et al.*, 2013 ; Musaad Mustafa *et al.*, 2013 ; Yang *et al.*, 2013 ; Hasni *et al.*, 2023).**

The registrated amount of sugar in the DU samples is possibly resulting from browsing the desertic plant containing a high polysaccharides amount such as cellulose **(Halas *et* Babinszky, 2014 ; Lojkova *et al.*, 2020)**. In fact, in 2017, Alhaider *et al.,* carried a GC-MS characterization on the camel urine, where they declared the existence of some saccharides' compounds such as galactose, D-galactose, melibiose, and 2-Deoxy-galactose released from glycolysis metabolic ways. These results confirm the unique functionality of the *Cumulus dromedaries'* kidneys comparing to the other mammals **(Tharwat, 2020)**.

The sugar compound concentration in both formulas corresponds perfectly with the eliminated amount of lactose and the added percentage of the saccharides from the urine, highlighting the absence of molecular interactions between the sugary elements of both

liquids. Furthermore, the acquired values in both mixtures confirm that the scanning calculation using FTIR method is very effective for the sugar quantification in dairy products and biological liquids **(Anjos *et al.*, 2015 ; Yaman *et* Durakli Velioglu, 2019 ; Hong *et al.*, 2021)**.

The DMU1 and DMU2 sugar content match with the amount recorded in both liquids separately, eliminating the possible molecular interactions including the sugar elements between DM and DU's molecules.

The documented percentage of lipids in the DM samples was within the fats average cited by many works on this milk type **(Khaskheli *et al.*, 2005 ; Brezovečki *et al.*, 2015 ; Yoganandi *et al.*, 2015, Zibaee *et al.*, 2015 ; Singh *et al.*, 2017 ; Ho *et al.*, 2021 ; Swelum *et al.*, 2021 ; Oselu *et al.*, 2022)**. However, the declared amount count to be low in the general level in this milk **(Singh *et al.*, 2017 ; Ho *et al.*, 2021 ; Swelum *et al.*, 2021 ; Oselu *et al.*, 2022)**.

In addition to proteins and sugar, the fat content is extremely affected by the food consumed quantity which unfortunately is very limited in the selected season where few plants are founds to fed these animals **(Musaad Mustafa *et al.*, 2013 ; Yang *et al.*, 2013 ; Swelum *et al.*, 2021)**.

The notable decrease in the fat concentration of the both mixtures mainly resulted from the removed volume of milk which was replaced by 10% of urine in the DMU1 and 20% of urine in the DMU2.

III.1.2.2. Mineral content

Chloride (Cl), sodium (Na), potassium (K) and calcium (Ca) contents in dromedary milk, urine, DMU1 and DMU2 are presented in figure 16. The minimum and maximum amounts of those elements are illustrated in table VIII.

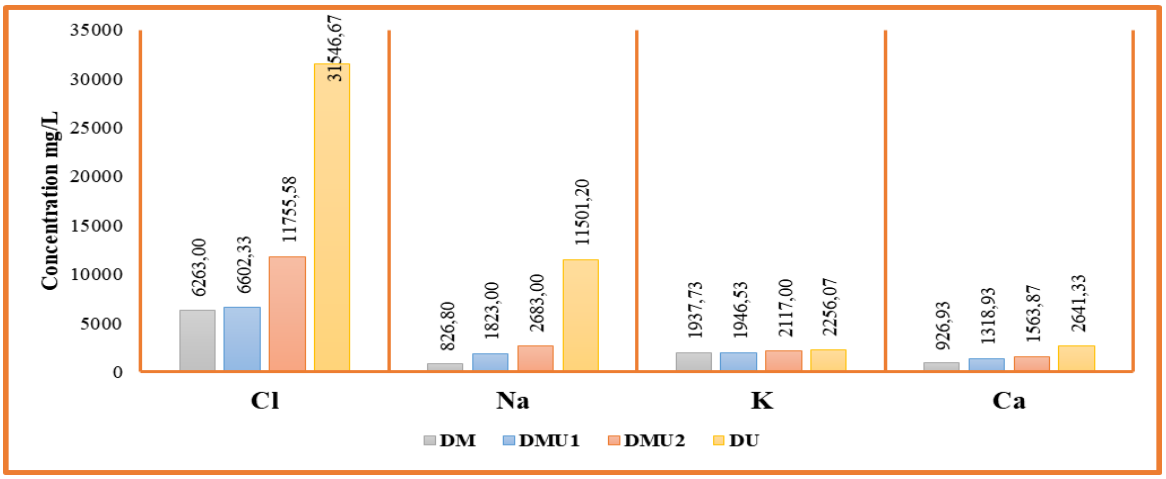

Figure 16. Mineral content of dromedary milk, urine and the two formulas.

The four tested samples showed a very important content in the investigated minerals, where the highest amount was registrated by the chloride in all the tested samples, precisely by the DU that recorded a concentration of 31546.667±2.318 mg/l, followed by the DMU2, DMU1 and DM with a content of 11755.58±115.79, 66263 ±2.27 and 6602.330±13.20 mg/l, respectively.

Moreover, DU registrated also the greatest amount in the Na, Ca and K with concentrations of 11501.200±1.31, 2641.333±3.2 and 2256.067±4.08 mg/l, respectively. In the other side, the lowest amount of those minerals was revelated in the milk samples at 826.8±2.51 mg/l sodium, 1937.73±2.34 mg/l potassium and 926.93±116 mg/l calcium (Figure 16).

The increasing of the mineral content in both formulas was proportional to the urine added quantity (Figure 16), (Table VIII). Indeed, the DMU2 showed a higher content in the investigated elements comparing to the DMU1, where it registrated an amount of 2683.000±1.31, 2117.000±1.249 and 1563.867±1.665 mg/l for the Na, K and Ca, respectively. While the DMU1 samples showed concentrations of 1823.000±6.470 mg/l for the Na, 1946.533±4.01 mg/l for the K and 1318.930±2.91 mg/l for the Ca.

As demonstrated in table VIII, a great variation was noted between the minimal and maximal concentration of all the quantified minerals in all the samples reflecting a very significant difference ($p<0.05$).

Table VIII. Mineral content of dromedary milk, urine and their mixtures.

		DM	DMU1	DMU2	DU
Cl	**Min**	4342.667±3.215$^{(c)}$	4806.670±0.577$^{(d)}$	6543.000±2.646$^{(b)}$	10873.333±3.055$^{(a)}$
	Max	8852.667±2.517$^{(c)}$	8386.670±0.577$^{(d)}$	19198.667±576.486$^{(b)}$	45380.333±0.577$^{(a)}$
	Mean	6263.000±2.272$^{(c)}$	6602.330±13.200$^{(d)}$	11755.580±115.796$^{(b)}$	**31546.667±2.318$^{(a)}$**
Na	**Min**	739.667±2.887$^{(c)}$	1670.000±5.196$^{(d)}$	2301.667±2.082$^{(b)}$	8875.000±5.000$^{(a)}$
	Max	943.000±4.359$^{(c)}$	2115.670±1.069$^{(d)}$	3343.333±3.055$^{(b)}$	14012.333±10.970$^{(a)}$
	Mean	826.800±2.506$^{(c)}$	1823.000±6.470$^{(d)}$	2683.000±1.311$^{(b)}$	**11501.200±1.311**$^{(a)}$
K	**Min**	1723.000±1.732$^{(b)}$	1596.677±2.062$^{(d)}$	1623.667±1.528$^{(c)}$	1807.667±6.807$^{(a)}$
	Max	2185.333±5.033$^{(c)}$	2310.000±1.732$^{(d)}$	3051.333±1.528$^{(a)}$	2957.333±6.658$^{(b)}$
	Mean	1937.733±2.344$^{(c)}$	1946.533±4.005$^{(d)}$	2117.000±1.249$^{(b)}$	**2256.067±4.081**$^{(a)}$
Ca	**Min**	902.667±2.517$^{(c)}$	1125.333±1.102$^{(d)}$	1351.667±1.528$^{(b)}$	2182.000±6.557$^{(a)}$
	Max	1093.000±36.592$^{(c)}$	1553.333±2.082$^{(d)}$	1853.667±3.215$^{(b)}$	2680.333±4.509$^{(a)}$
	Mean	926.933±116.002$^{(c)}$	1318.930±2.910$^{(d)}$	1563.867±1.665$^{(b)}$	**2641.333±3.202**$^{(a)}$

$^{a-d}$: Different letters in the line indicate a significant difference (P <0.05) between the results of different dromedary samples. DM: Dromedary milk, DMU1: Dromedary milk-urine mixture 9-1 (v/v), DMU2: Dromedary milk-urine mixture 8-2 (v/v), DU: Dromedary urine.

The recorded Ca concentration in the DM samples was withing the average cited by many previous studies that starts from 880 to 1500 mg/l **(Zibaee *et al.*, 2015 ; Mostafidi *et al.*, 2016 ; Benmeziane–Derradji *et* Production, 2021 ; Konuspayeva *et al.*, 2022)**. However, this mineral documented a significant variation either between the tested DM samples or in the precedent works. Indeed, due to the physiological state of the animal and the food and water consummation, all declared Ca content in this milk couldn't be limited in narrow interval **(Zibaee *et al.*, 2015 ; Mostafidi *et al.*, 2016 ; Benmeziane–Derradji *et* Production, 2021 ; Konuspayeva *et al.*, 2022 ; Hasni *et al.*, 2023).**

Besides Ca, Na and K concentrations can also be influenced by the animal general state such as the lactation stage and age, browsed plants and water intake **(Musaad Mustafa *et al.*, 2013 ; Mostafidi *et al.*, 2016 ; Konuspayeva *et al.*, 2022)**. Thought, the registrated content of both minerals was comparable to the ones stated by precedent works. In point of fact, the general average of the two electrolytes includes the registrated concentrations of the tested samples **(Gorban *et* Izzeldin, 1997 ; Sharma *et al.*, 2014 ; Yoganandi *et al.*, 2015 ; Mostafidi *et al.*, 2016 ; Sumaira *et al.*, 2020 ; Konuspayeva *et al.*, 2022).**

Contrary to other three minerals, the chlorides content is not well investigated in dromedary milk and urine, where limited works were carried on the determination of its concentration. Based on the very little cited works, the chlorides general amount in this milk

type was found with a mean of 2500 mg/l **(Inayat *et al.*, 2003 ; Khaskheli *et al.*, 2005 ; Yoganandi *et al.*, 2015 ; Ismaili *et al.*, 2019 ; Konuspayeva *et al.*, 2022)**, a value was two times lower than the one documented in this work. A difference mainly caused by the feeding system **(Kashaninejad *et* Razavi, 2021)**.

Dromedary milk count to be a reach source of Ca, Na and K, guaranteeing the needed amount to the human body for the consumers **(Konuspayeva *et al.*, 2008 ; Mostafidi *et al.*, 2016)**.

The urines samples recorded a very important amount of the four minerals. However, because unavailability of precedent data, none of the obtained minerals' concentrations can be evaluated. In fact, so far dromedary urine couldn't take a place to be perfectly studied and characterized. Therefore, the founded amounts of Ca, Na, K and Cl are related in a direct way to kidneys secretion and water consummation **(Abdalla, 2020 ; Alim *et al.*, 2019)**. Indeed, it was declared in multiple researches that the undergrounded water of the desertic regions is highly saturated with the minerals, especially chlorides that reached the content of 5000mg/l in the southern regions of Algeria **(Bouchekima *et al.*, 2008 ; Aghazadeh *et al.*, 2017 ; Saberimehr *et al.*, 2017 ; Singh *et al.*, 2021)**.

The Ca, Na, K and Cl content increasing in both formulas can negatively affect the milk constituent especially the proteins by interacting with their amino acids which can eventually lead to a structural modification. Moreover, it has been proved that the high concentrations of menials elements in milk reduce proteins solubility which increase their coagulation **(Pérez-Llamas *et al.*, 2001 ; Schmitt *et al.*, 2011 ; Ocak *et* Rajendram, 2013)**.

The augmentation of minerals content in the DMU1 and DMU2 can influence the structure and solubility of milk proteins.

III.1.2.3. SDS-PAGE characterization

The separation patterns of the milk proteins of the DM, DMU1 and DMU2 in addition to the DU are shown in figure 17.

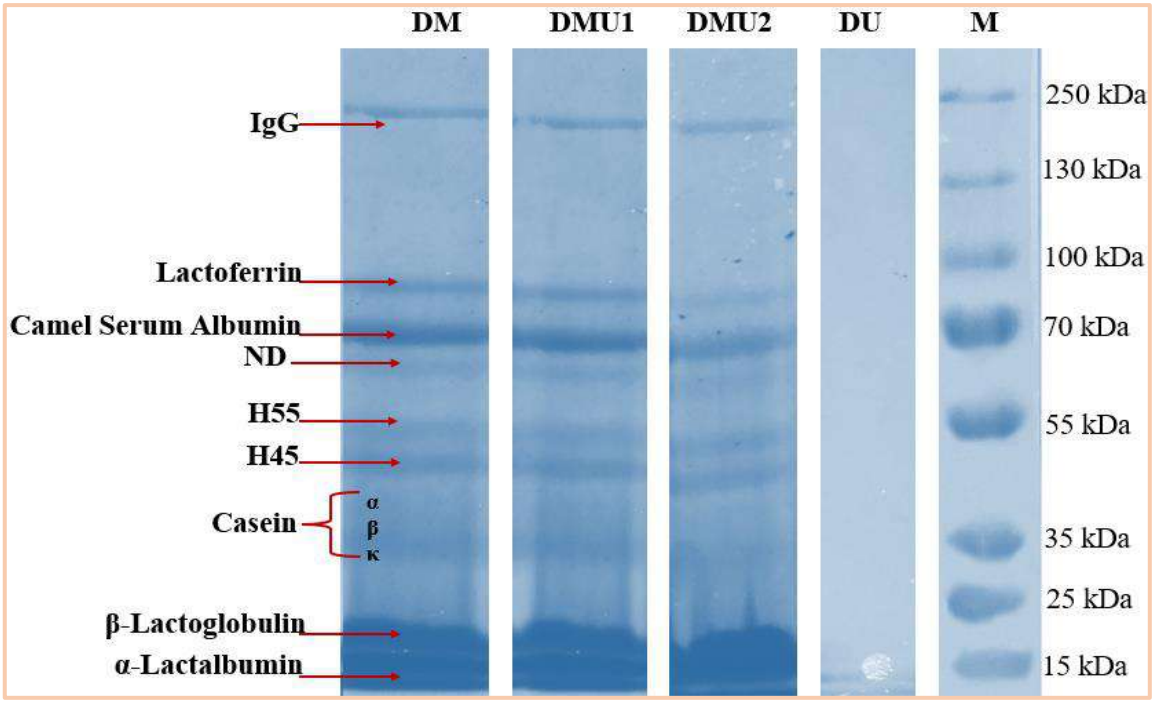

Figure 17. Separation patterns of camel milk proteins on SDS-PAGE, DM: Dromedary milk, DMUA: Dromedary Milk-Urine mixture 9-1 (v/v), DMU2: Dromedary Milk-Urine mixture 8-2 (v/v), DU: Dromedary urine.

The separation pattern of the samples containing milk on the gradient from 7.5 to 17.5 % showed the presence of 9 proteins bands corresponding to the dromedary milk proteins, with a molecular weight ranged from 210 kDa to 14kDa (Figure 17). The DU Lane exhibits a total proteins absence even with a small molecular weight. Neverless, milk and milk incorporated with urine samples present almost identical bands appearance. The more milk is eliminated the less the DMU1 and DMU2 lanes are brighter and the band is a bit lower than the one of DM proteins (Figure 17).

Nine major bands were appeared after proteins' electrophoresis migration in the DM, DMU1 and DMU2's lanes. The band with the highest molecular weight represents the immunoglobulin G (IgG) with a wight of 210KDa **(Shuiep, 2015 ; Yasmin *et al.*, 2020)**. Furthermore, most of the antimicrobial proprieties of dromedary milk are due to the IgG proteins **(Konuspayeva *et al.*, 2007 ; Singh, 2011 ; Makkawi *et al.*, 2019 ; Swelum *et al.*, 2021)**.

The second band with a heaviness of ≈87KDa reflects the lactoferrin protein, followed by the camel serum albumin (CSA) with a mass of ≈68KDa **(Shuiep, 2015 ; Yasmin *et al.*, 2020)**. The two bans exhibiting a molecular mass of 55 and 45 are mainly related to the

immunoglobulins G's heavy and light chains **(Shuiep, 2015 ; Akindykova *et al.*, 2019 ; Yasmin *et al.*, 2020)**.

Nevertheless, one large dark band was appeared with a molecular weight ranging from ≈37kDa to ≈25kDa representing exclusively the three-milk casein α, β and κ (α-CN, β-CN and κ-CN) that documented to weighs 36KDa, 31KDa and 25KDa, respectively **(Kappeler, 1998 ; Omar *et al.*, 2016 ; Akindykova *et al.*, 2019 ; Mahala *et al.*, 2022)**. The three caseins represent the dominant category with a percentage that varies between 75% and 80% of the total DM's proteins **(Mudgil *et al.*, 2022 ; Singh *et al.*, 2017)**. In which, the β-CN consist of 65% of the DM's caseins, while the α-CN account for 31%. The κ-CN represent only 4% of this protein category (**Akindykova *et al.*, 2019 ; Singh *et al.*, 2017 ; Mahala *et al.*, 2022 ; Mudgil *et al.*, 2022**).

The bands with fastest mobility and the lower mass reflect the α-Lactalbumin that varies between 18kDa and 14kDa as molecular wight **(Yasmin *et al.*, 2020)**. A protein that consists almost 50% of the total whey which represents an abondance of 20-25% of the total dromedary milk proteins **(Mudgil *et al.*, 2022 ; Singh *et al.*, 2017)**.

Guarding the molecular weight doesn't ensure the structural modification, indeed many papers showed that proteins keep their mass despite the structural changing **(Bao, 2009 ; Counts, 2010 ; Artigues *et al.*, 2016)**. Therefore, the SDS-PAGE characterization cannot confirm the presence of a molecular interaction between the DU's constituent with the DM proteins and their possible oxidation caused by the urine addition **(Wittig *et* Schägger, 2005).**

III.1.2.3. Protein oxidation

The mean value of the free thiols groups concentration resulting from the proteins oxidation are represented in figure 18.

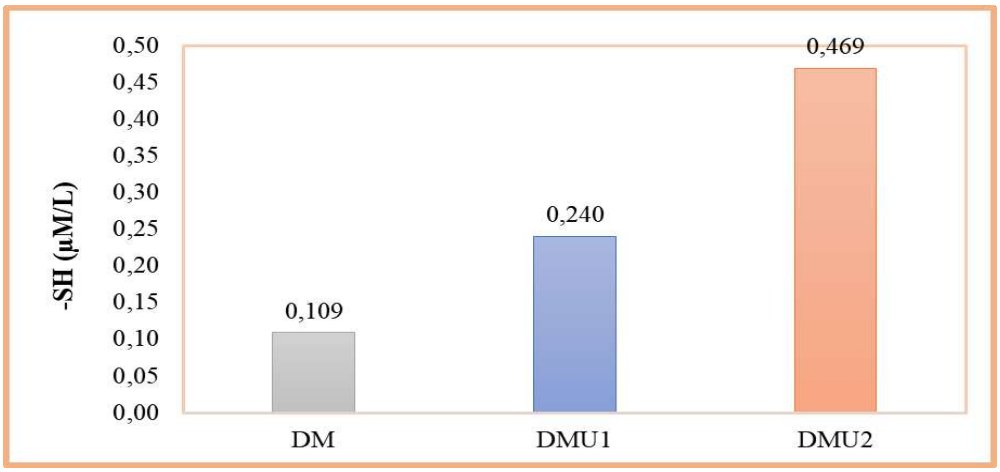

Figure 18. Sulfhydryl groups resulted from proteins oxidation in DM, DMU1 and DMU2.

As reveals by the above figure, the DMU2 sample recorded the highest free sulfides' groups concentration that was 0.469 µM/l, a concentration two times higher than the one demonstrated by the DMU1 and four-time greater comparing to the amount present in the DM.

The DM samples oxidation could be caused by oxygen active species produced usually from the reaction of the milk constituent with the light or other factors **(Hellwig, 2019 ; Kurz *et al.*, 2020).**

Furthermore, it was documented from the obtained results that the concentration of the -SH group was increasing with the urine addition. In fact, the more urine is added, the free thiols are produced. The urine compounds somehow can cause the breakage of the SS bound between the milk proteins' amino acids.

A protein oxidation could be resulting from the direct interaction between the urine elements with the DMs' proteins, changing the general proprieties of the milk such as the augmentation of the pH level or increasing the mineral content, both factors can lead to expose the SS linkages leading to their dissociation **(Shacter, 2000 ; Baba *et* Bhatnagar, 2018 ; Hellwig, 2019 ; Xiong *et* Guo, 2021)**. Nevertheless, the urine molecules can enhance milk proteins oxidation by playing the intermediate agents role. Indeed, it has been reported that most of the oxygen reactive species needs an intermediate, so it can react with the proteins to be oxidize, what the urine offers **(Hellwig, 2019 ; Niero *et al.*, 2015)**.

Proteins functionality, mobility and solubility are sustained due to their general structure which is preserved by the amino acids linkages as the S-S bounds **(Feige *et al.*,**

2018, Sun *et al.*, 2004, Zhou *et* Pang, 2018). Breaking the thiols links lead to modifying the general structure of the milk proteins which in returns influence their general functionality by exposing their hydrophobic domain **(Gharbi *et al.*, 2022, Sadiq *et al.*, 2021, Trivedi *et al.*, 2009).** Some reactive groups enhancing by that the rheological and physiological proprieties of the milk **(Hawkins *et* Davies, 2019)**.

Nevertheless, the oxidation of the protein's -SH groups decrease their hydrolysis by peptic enzymes. In fact, the digestibility of the milk casein can dropdown to 80% in case of the thiol groups oxidation **(Xiong *et* Guo, 2020)**. Most of the dromedary milk therapeutical forces are guaranteed by the bioactive peptides released from the digestion of the DM's proteins **(Brandelli *et al.*, 2015 ; Zibaee *et al.*, 2015 ; Marcone *et al.*, 2017 ; Guha *et al.*, 2021; Swelum *et al.*, 2021)**. The more proteins are digested the more peptides are produced and the more activities are offered by this milk **(Marcone *et al.*, 2017 ; Beverly *et al.*, 2019 ; Giromini *et al.*, 2019 ; Guha *et al.*, 2021)**. Unless, the proteins are oxidized, where pepsin, trypsin and chymotrypsin cannot access to the exact breaking point reducing or modifying by that the generated peptides which can be caused by the urine e addition **(Feng *et al.*, 2015 ; Li *et al.*, 2019 ; Guha et al.,2021).**

The addition of the urine to the milk causes proteins oxidation affecting their functionality and digestion.

III.1.2.4. Statistical analysis

The results of principal component analysis (PCA) that was carried out with the Excel software (XLSTAT 2016.02.28451) are illustrated in figure 19 and figure 20.

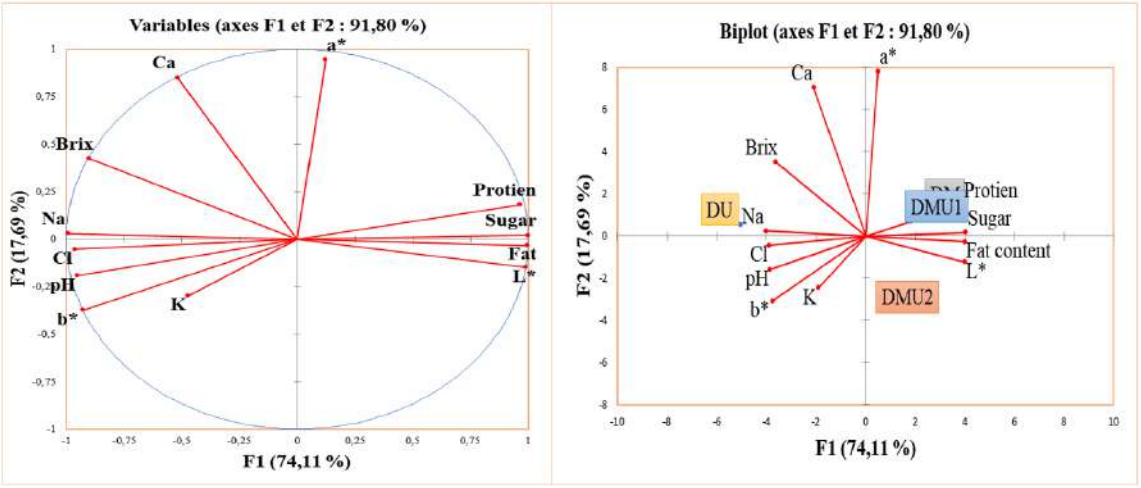

Figure 19. Principal component analysis (PCA) of variables results

The analysis results of the four different samples, through the investigated variables that were the physical, chemical and biochemical characteristics demonstrate the existence of a very importance difference between all the tested samples. Where, the difference between the two axes variable F1 and F2 (91.80%) was higher than 70% (Figure 19).

Basing on gathering the variance and biplot analysis, both axes observation on a 91.80 level shows a rapprochement and similarity between the DM and DMU1 in the general characteristics, while notable distinct and dissimilarity was observed between DU, DMU2 and the two other samples (Figure 19).

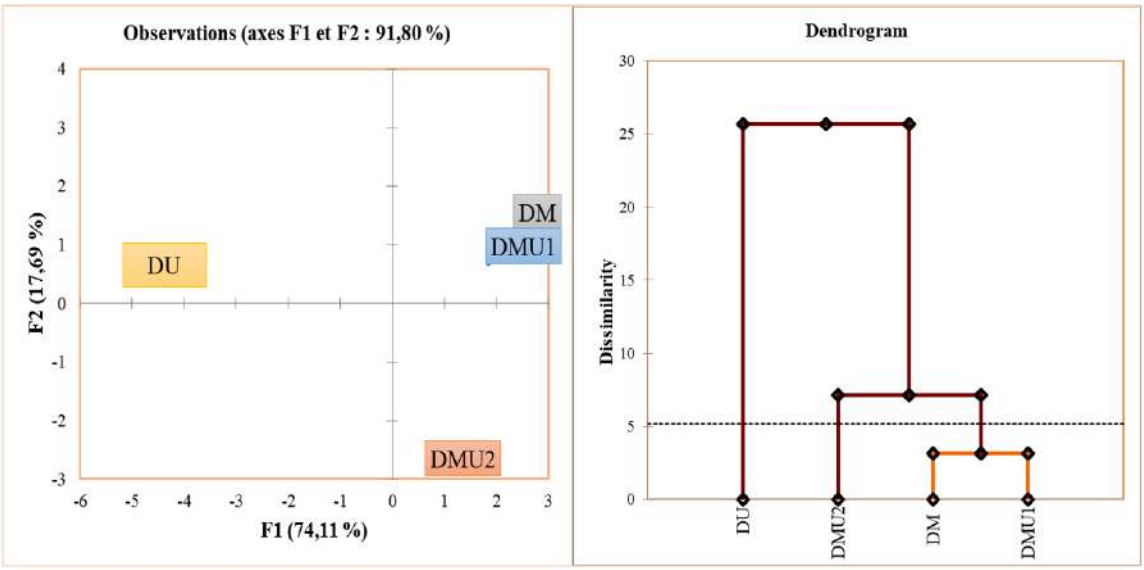

Figure 20. Principal component analysis observation results.

Indeed, the final analysis results that appears in the dendrogram (Figure 20), exhibit the ascending hierarchical classification that divides the four tested samples in three major classes as following:

- The first class, also divided in two subclasses containing DM and DMU1 samples;
- the second class is represented by DMU2 samples;
- the third class concerns the DU samples.

III.2. Hydrolysates characterization

III.2.1. Hydrolysates characterization with SDS-PAGE

The samples SDS-PAGE pattern in 7.5-20% gradient before and after the *in vitro* digestion process is represented in figure 21.

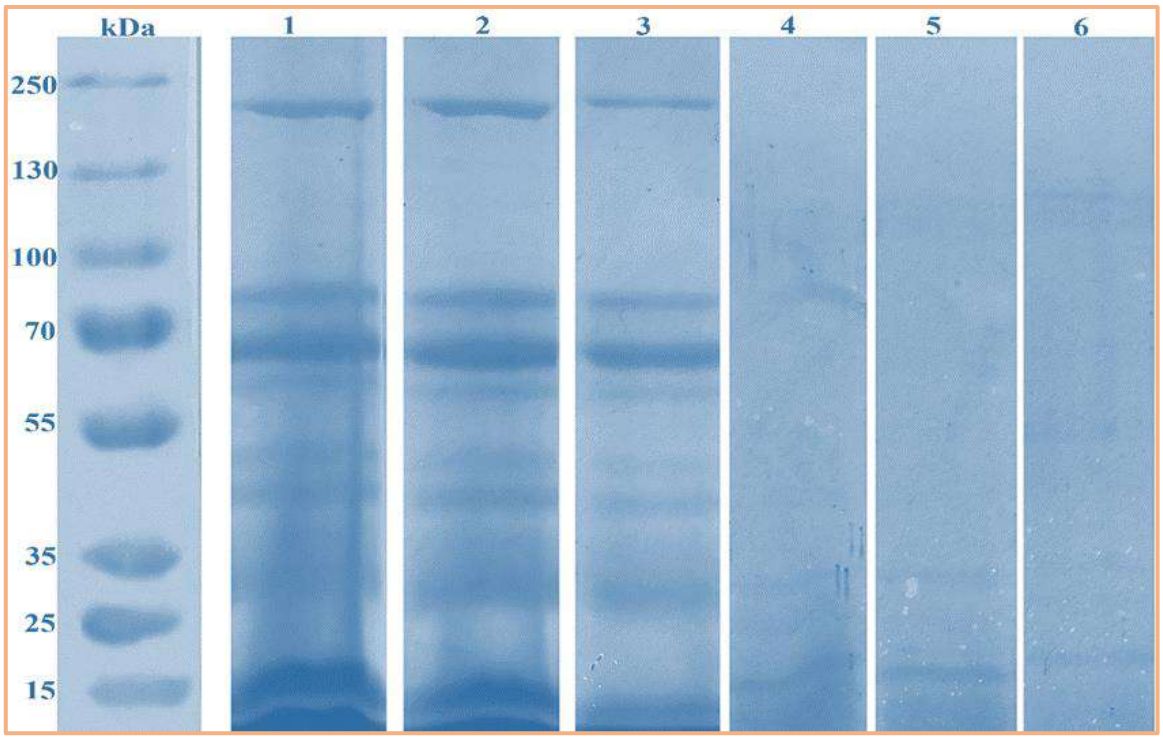

Figure 21. Separation patterns of dromedary milk proteins on SDS-PAGE before and after the *in vitro* hydrolysis. Lane 1: DM, Lane 2: DMU1, Lane 3: SMU2. Lane 4,5 and 6 reflect the patterns of the corresponding hydrolysates by peptic enzymes, Lane 4: DMH, Lane 5 and 6: DMUH1 and DMUH2, respectively.

The digested samples electrophoresis before the hydrolysis operation on the mentioned gradient represented in lane 1, 2 and 3 show the presence of all the dromedary milk proteins. After the degradation by the peptic enzymes' pepsin and trypsin, the digested samples pattern (lane 4, 5 and 6) clearly shows the an most disappearance of the dromedary milk proteins that was apparently degraded into smaller peptides with a molecular weight less than 14kDa and probably into free amino acids (Figure 21). Nevertheless, these results point that the DU compounds doesn't influence and inhibits both peptic enzymes activities.

The digested samples migration in the 7.5-30% gradient is shown in figure 22.

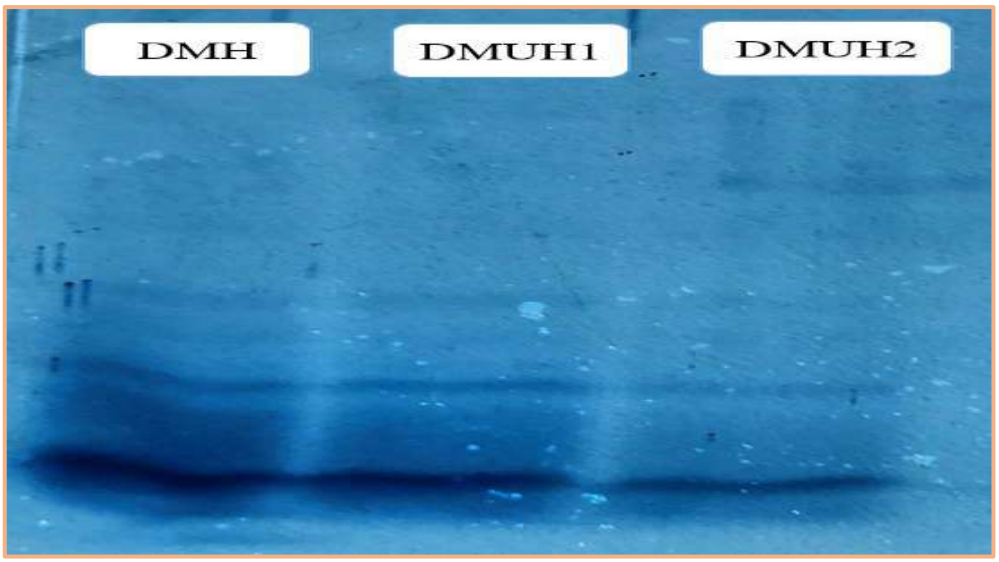

Figure 22. Separation patterns of dromedary milk proteins on SDS-PAGE after the hydrolysis process on a 7.5-30% gradient.

The profile of SDS-PAGE migration at 7.5-30% gradient confirm the previous remark, where the existed proteins in DM, DMU1 and DMU2 were hydrolyzed in peptides with a low molecular weight. Indeed, samples' peptides released from the digestion operation are trapped at the end of the gel where the halls are very small allowing their visualization (Figure 22).

Moreover, the above figure also reveals a color distinction between the three samples. The lane containing the DMH peptides appear with a more intense color comparing to the two mixtures hydrolysates. the more the milk is removed the less the lane color is intense and the less peptides content exist in the DMUH1 and DMUH2 (Figure 22).

III.2.2. Hydrolysates characterization with FTIR

Absorbance FTIR spectra of DMH, DMUH1, DMUH2 and DUH are represented in figure 23.

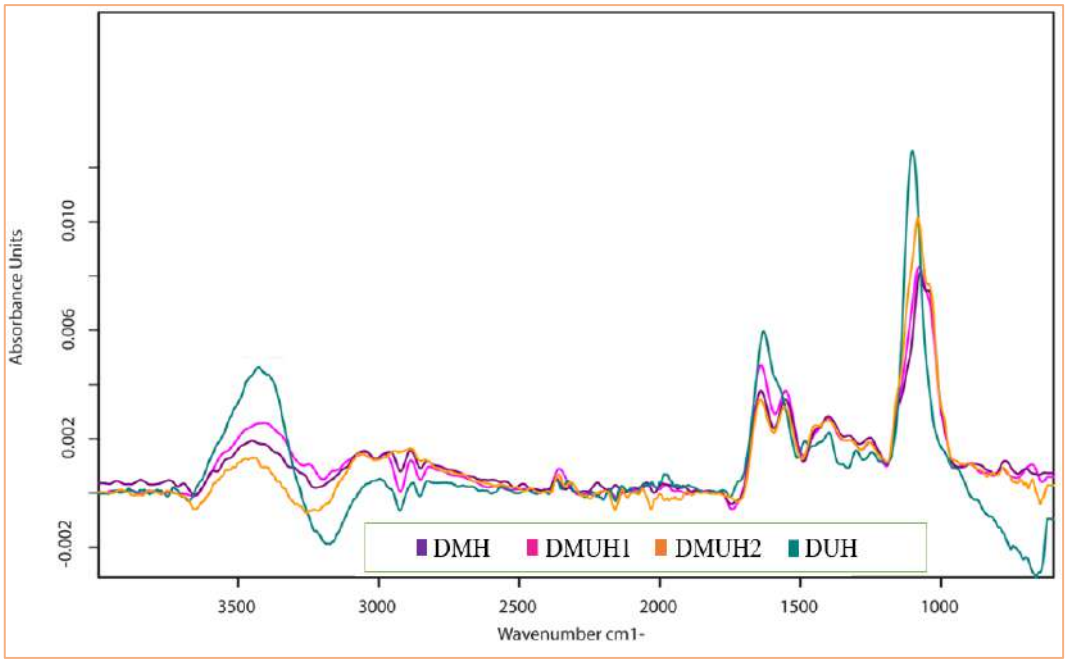

Figure 23. FTIR spectra of the hydrolysates

The four hydrolysates' samples exhibited a similarity in their FTIR profile, especially between the three digested samples containing milk. In fact, DMH, DMUH1 and DMUH2 showed almost identical peaks at the same wavenumber. However, a remarkable dissimilarity in the absorbance intensity was observed between those three samples (figure 23).

The scanning ATR-FTR profile of the DMH exhibit the highest peak at ~ 1080 cm^{-1} that is related to the carbohydrate group "C-O" corresponding to the lactose **(Aernouts *et al.*, 2011 ; Mesgaran *et al.*, 2020)**. Following by medium peaks at 1250cm^{-1} and 1330cm^{-1} reflect the stretching of the C-N group and at 1410 cm^{-1} of the S=O group **(Ng and Simmons, 1999 ; Stuart, 2004 ; Weyer, 2007 ; Thompson, 2018)**.

The amide I and amide II peaks appeared in order at ~1642 and ~1550 due to the existence of peptides **(Stuart, 2004 ; Grewal *et al.*, 2018 ; Markoska *et al.*, 2019)**. A large band at ~ 3500 cm^{-1} belongs to the hydroxyl group "OH" and the peptides and amino acids N-H groups **(Weyer, 2007 ; Grewal *et al.*, 2018 ; Thompson, 2018 ; Guha *et al.*, 2021)**. Other peaks were noted at ~770cm^{-1} indicating the presence of aromatic rings relating to the amino acids like tryptophan or to phenolic compounds. A very small peak at ~2929cm^{-1} belongs to the "CH" aliphatic group and fatty acids acyl chain **(Grewal *et al.*, 2018 ; Ho *et al.*, 2019 ; Deshwal *et al.*, 2022 ; Hasni *et al.*, 2023,)**.

The DUH FTIR scan showed a large medium peak at 3440cm^{-1} indicating the OH bound and the N-H stretching caused by the urea. Moreover, a medium band was produced at ~1630cm^{-1} due to the NH amine group and the C=O group stretching of the urine nitrogenous and carbonyl elements such as urea, uric acid and creatinine **(Carnevalli *et al.*, 2019 ; Algethami *et al.*, 2020 ; Sarigul *et al.*, 2021)**. However, the observed peaks were not very high reflecting a low concentration of those elements in the DU comparing to FTIR profile of other mammals and human urine **(Algethami *et al.*, 2020 ; Sarigul *et al.*, 2021)**.

The two bands at 1479cm^{-1} and 1398cm^{-1} would be due to OH bending and the peaks appearing at 1300cm^{-1} and 1246cm^{-1} caused by the presence of the CH bending group **(Stuart, 2004 ; Weyer, 2007 ; Grewal *et al.*, 2018 ; Sarigul *et al.*, 2021).**

Nevertheless, a very strong sharp peak at ~1100cm^{-1} represents the stretching of the CO, CH and C-N-C groups in addition to the PO, NH2 and SO stretching due to the presence of urea, sulfate, creatinine, phosphate and nucleic acid **(Carnevalli *et al.*, 2019 ; Algethami *et al.*, 2020 ; Sarigul *et al.*, 2021)**.

Results exhibit that hydrolysate of DMH and DMUH1 as well as DMUH2 mixtures showed almost similar ATR-FTR profile. Yet, a close examination can illustrate slight deference between the DMH pattern and the DMUH mixtures patterns with a variation of 2 to 4 cm^{-1} in each major peak. This deference can be due to the molecular interaction between each liquid that can slightly influence the stretching of the molecular groups **(Weyer, 2007 ; Grewal *et al.*, 2018 ; Thompson, 2018)**.

Furthermore, optical density of the three milk hydrolysates revealed clear deference in their functional group concentration, explained by the decrease of the milk amount in addition to the molecular interaction or new bounds formation between the milk and the urines compounds.

The FTIR analysis point the existence of molecular interaction between the DMH and DUH substances.

III.2.3. Hydrolysates characterization with HPLC-DAD

Qualitative and quantitative detection of phenolic compounds in the tested hydrolysates are consigned in table IX.

Table IX. Individual phenolic compounds of digested samples.

Phenolic compounds	WL (nm)	DMH		DMUH 1		DMUH 2		DUH	
		C (mg/l)	RT	C (mg/l)	RT	C (mg/l)	RT	C (mg/l)	RT
Gallic acid	278	7.591±0.02$^{(aA)}$	6.534	7.018±0.32$^{(bB)}$	6.456	7.194±0.21$^{(abAB)}$	6.51	0.000$^{(cC)}$	NPD
Protocatechuic	254	5.791±0.66$^{(aA)}$	9.241	5.547±0.34$^{(aA)}$	9.29	5.955±0.13$^{(aA)}$	9.355	6.556±0.77$^{(aA)}$	9.43
4-hydroxybenzoic acid	254	0.267±0.01$^{(bB)}$	13.767	0.089±0.00$^{(cC)}$	1.,23	0.028±0.00$^{(cC)}$	13.041	0.927±0.08$^{(aA)}$	13.016
Caffeic	280	10.646±0.52$^{(bB)}$	14.985	15.961±2.30$^{(aA)}$	15.124	10.832±0.19$^{(bB)}$	15.142	14.296±0.84$^{(aA)}$	15.087
Syringic	278	0.519±0.03$^{(cC)}$	16,224	5.361±0.50$^{(bB)}$	16.244	0.989±0.05$^{(cC)}$	16.2	18.012±1.61$^{(aA)}$	15.471
trans 4 Hydroxycinnamic	320	0.182±0.02$^{(bB)}$	19.403	0.165±0.00$^{(bB)}$	20.192	0.166±0.00$^{(bB)}$	20.146	0.302±0.07$^{(aA)}$	20.157
Rutin	255	10.160±1.35$^{(cC)}$	20.675	22.401±2.52$^{(bB)}$	20.244	30.811±1.08$^{(aA)}$	20.173	9.099±0.96$^{(cC)}$	21.149
Ferulic acid	320	0.000$^{(bB)}$	NPD	0.731±0.01$^{(aA)}$	22.448	0.714±0.01$^{(aA)}$	22.458	0.705±0.03$^{(aA)}$	22.421
Trans 3 hydroxy	278	2.635±0.29$^{(bB)}$	23.712	2.688±0.07$^{(bB)}$	23.629	2.527±0.03$^{(bB)}$	23.789	4.979±0.61$^{(aA)}$	23.43
D-coumaric	320	0.000$^{(cC)}$	NPD	0.000$^{(cC)}$	NPD	0.392±0.01$^{(bB)}$	25.616	0.451±0.03$^{(aA)}$	25.399
Myricetin	360	0.000	NPD	0.000	NPD	0.000	NPD	0.000	NPD
Quercetin	360	10.811±0.13$^{(aA)}$	34.144	10.487±0.04$^{(bB)}$	31.834	10.592±0.02$^{(bB)}$	31.898	10.483±0.09$^{(bB)}$	31.767
Kaempferol	360	2.974±0.05$^{(aA)}$	35.866	2.027±0.00$^{(bB)}$	36.254	2.082±0.02$^{(bB)}$	36.331	2.095±0.05$^{(bB)}$	36.19
Chrysin	254	2.691±0.02$^{(bB)}$	41.469	0.751±0.01$^{(cC)}$	40.967	0.738±0.00$^{(cC)}$	41.393	4.965±0.27$^{(aA)}$	41.379

WL: wavelength**, RT:** Retention Time, **C:** Concentration (mg/L) and **NPD**: No Peak is Detected.

a-c: Different letters in the column indicate a significant difference (P <0.05) between the results of same camel samples; A-C: Different letters in the line indicate a significant difference (P <0.05) between the results of different

The HPLC analysis allowed a qualitative and quantitative determination of some phenolic compounds in the four samples hydrolysates, where thirteen secondary metabolites were detected. Among the revealed phenols, eleven elements were found in the DMH, in which caffeic, rutin and quercetin were the most abandoned with concentrations exceeding slightly the 10 mg/l. Contrary to the 4-hydroxybenzoic acid, syringic and trans-4-hydroxycinnamic that were present in the lowest concentrations (Table IX).

Eleven secondary metabolites were also revealed in the DUH. The highest content at 18.01 mg/g, 14.29 mg/l and 10.84 mg/l recorded by syringic, caffeic and quercetin, respectively (Table IX).

The phenolics compounds amount in the two mixtures hydrolysates was not proportional to their concentration in the DMH and DUH separately, this could be resulted from the interaction of those element with other constituent of milk and urine after being mixed. The DMUH1 showed a high content in quercetin with 10.487±0.04 mg/l in the same range as both liquids separates. Caffeic with 15.961±2.30 mg/l, higher than the one record by the DMH and DUH. Rutin with 22.401 mg/l, a concentration twice higher than the one reported by the milk and urine hydrolysates (Table IX). The minimal content of the detected compounds in the DMUH1 was registrated by 4-hydroxybenzoic acid, trans 4 Hydroxycinnamic, ferulic acid and chrysin with a concentration of 0.089±0.00, 0.165±0.00, 0.731±0.01and 0.751±0.01mg/l, respectively (Table IX).

Furthermore, DMUH2 registered five phenolic compounds with low concentrations of trans-4-Hydroxycinnamic (0.166±0.00 mg/l), Ferulic acid (0.714±0.01mg/l), D-coumaric (0.392±0.01mg/l), Chrysin (0.738±0.00 mg/l). The 4-hydroxybenzoic acid (0.028±0.00mg/l) recorded the minimal concentration comparing to all the examined hydrolysates (Table IX).

The Saharan plant species browsed by the dromedary are the source of the detected phenolic compounds in both milk and urines hydrolysates. Those molecules are known by their biological and therapeutic properties such as antioxidant, antimicrobial and anti-inflammatory capacities **(Bouchouka *et al.*, 2012 ; Cicerale *et al.*, 2010 ; Vougat *et al.*, 2015 ; Li *et al.*, 2021)**. They prevent and help treatment of some health diseases as cardiovascular, neurodegenerative and certain types of cancer **(Cicerale *et al.*, 2010 ; Aguilera *et al.*, 2016 ; Aboul-Enein *et al.*, 2013 ; de Oliveira *et al.*, 2020 ; Pereira *et al.*, 2020)**. Benefits of these compounds are mainly due to the presence of hydroxyl groups and phenolic rings **(Sroka *et al.*, 2003 ; Zuo *et al.*, 2018 ; Minatel *et al.*, 2017 ; Chen *et al.*, 2020 ; Li *et al.*, 2021)**.

III.2.3. Hydrolysates zetapotential and mobility

Samples hydrolysates' ζ-potential is presented in figure 24.

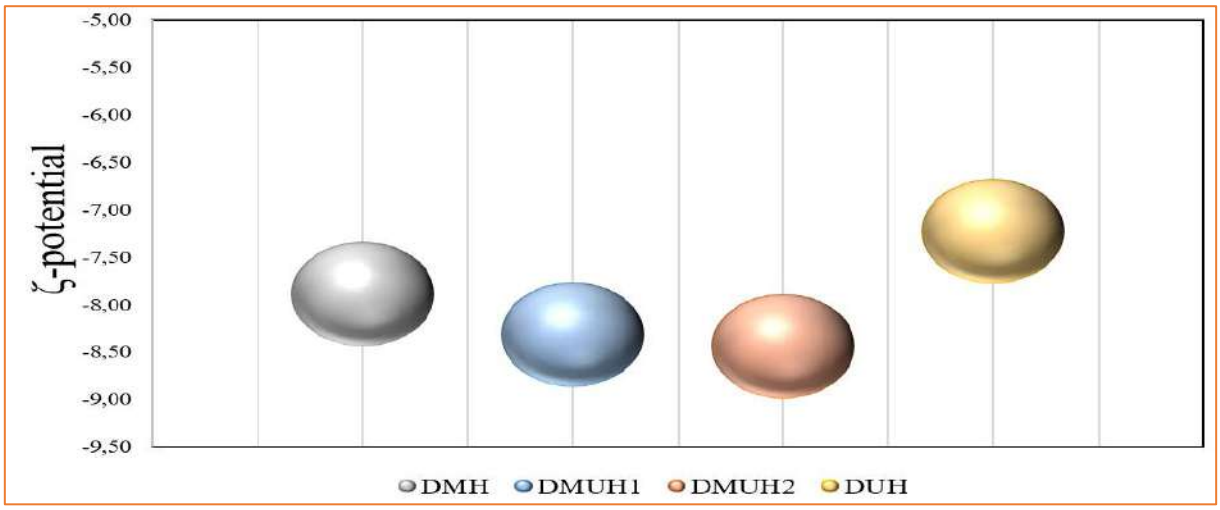

Figure 24. ζ potential of the hydrolysates

The molecules existing in the urine hydrolysates samples showed the higher ζ-potential that was -7.23 mV. The DMH ζ-potential of -7.89Mv was near to the DUH one. The values of the two digested mixtures potential were lower than the milk and urine hydrolysates. The DMUH1 documented a ζ-potential of -8.31 mV, bigger than the values registrated by the second mixture that was -8.44mV (Figure24).

The zeta-potential analysis also pointed the mobility of the molecules existing in the four hydrolysates as demonstrated in figure 25.

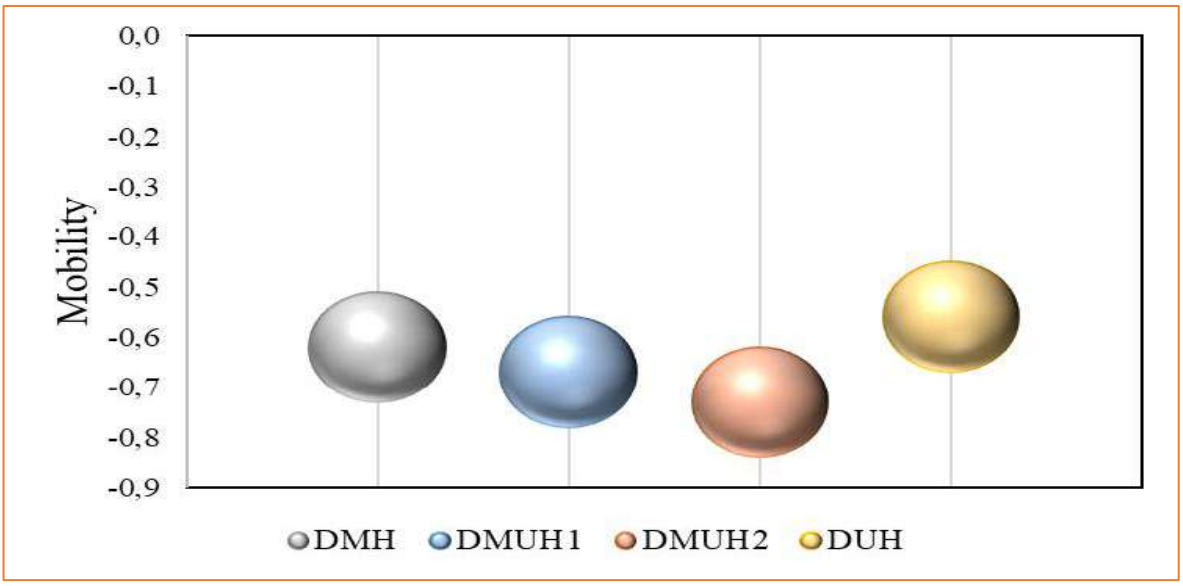

Figure 25. Hydrolysates molecules Mobility

The substances with the highest movement were found in the urine samples with a mobility average of -0.56±0.002µmcm/Vs. The DMH molecules also showed a mobility with a mean of -0.62±0.01µmcm/Vs. While, the two mixtures exhibited the lower mobility of -0.67±0.02µmcm/Vs for DMUH1 and -0.73±0.04µmcm/Vs for DMUH2 (Figure 25).

Most of the urine samples are characterized by small molecular weight giving there the swinging ability inside the liquid. Moreover, the changing in the pH level during the hydrolysis process can affect their ionization leading to a notable mobility due to the electronics interactions **(Kulsing *et al.*, 2015 ; Guo *et al.*, 2016 ; Malthouse, 2020 ; Abou-Diab *et al.*, 2021 ; Cruz-Casas *et al.*, 2021)**.

The DMH molecules movement can be associated directly to the released peptides and amino acids from proteins *in vitro* digestion. In fact, peptides and amino acids express deferent ionization stages depending on the pH, which was each time readjusted in the digestion experiment **(Fuierer *et al.*, 1994 ; Kulsing *et al.*, 2015 ; Guo *et al.*, 2016 ; Abou-Diab *et al.*, 2021)**.Therefore, their electronic case stimulates them to interact with each other for proton/electrons exchanging, producing their mobility **(Gunner *et al.*, 2006, Nocera, 2022)**.

The interaction between the urine and the milk hydrolysates substances allows the new bounds creation which lead to donation and receiving the needed electrons and protons, reducing in return their ionization. This interaction is the possible explanation of the decrease of the molecules mobility after incorporating the milk with urine.

The more urine is added, the less molecules are ionized and the lower electrophoretic mobility is expressed.

III.3. Therapeutical activities of hydrolysates

III.3.1. Acetylcholine esterase inhibition capacity

Hydrolysates half-maximal inhibitory concentration (IC_{50}) against the acetylcholine esterase (AChE) activity is represented in figure 26.

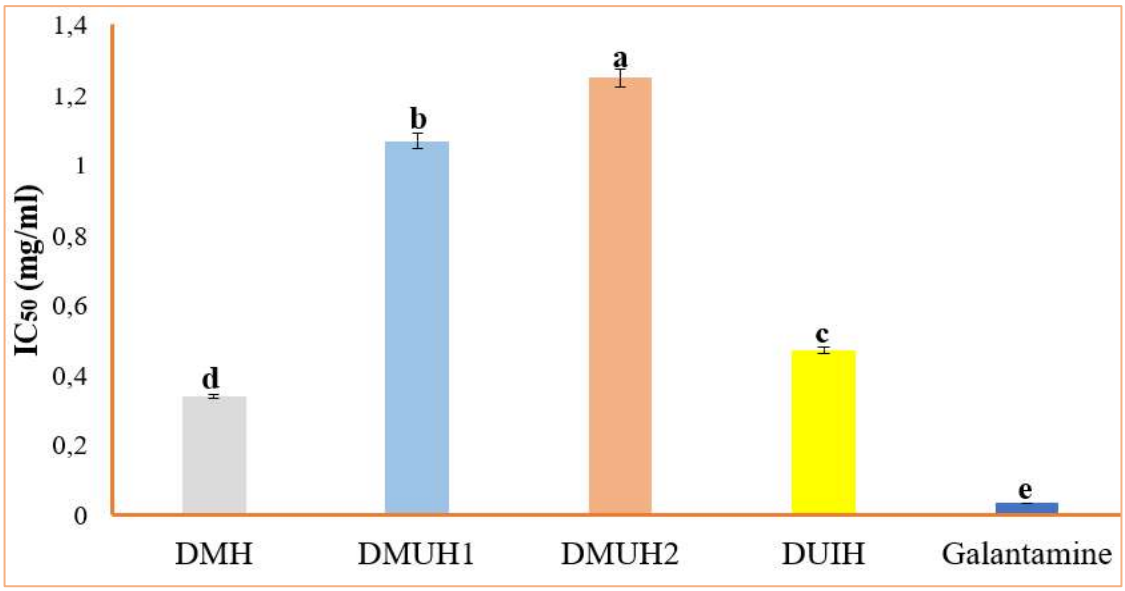

Figure 26. Hydrolysates half-maximal inhibitory concentration (IC_{50}) against the AChE activity

a-c: Different letters in the column indicate a significant difference ($P < 0.05$) between the results of same

An important inhibitory capacity to reduce the AChE activity was shown by the four hydrolysates. DMH sample recorded the lower IC_{50} of 0.341±0.039mg/ml reflecting the highest ability to block the AChE activity, followed by the urine samples that also presented an important inhibition capacity expressed by a low IC_{50} (0.472±0.074mg/ml) (Figure 25). The two formulas registrated an IC_{50} almost four times higher than the one of DMH and three times greater than the IC_{50} of the DUH sample. However, DMUH1 was a bit more active than the second formula hydrolysate, where the DMUH2' IC_{50} was slightly higher than the one of DMUH1 (Figure 26).

Galantamine which is the positive control, demonstrated the minor half-maximal inhibitory concentration comparing to the four hydrolysates with an IC_{50} of 0.037±0.002 which was ten time lower than the DMH IC_{50}, fourteen time minor than the DUH IC_{50} and thirty-two time inferior to the two mixtures IC_{50} (Figure 26).

The ability to inhibit the AChE activity demonstrated by DMH and the two mixtures hydrolysates can be related to the presence of bio-active peptides and amino acids generated by the *in vitro* digestion of the DM proteins. This mechanism can happen when the active site of this enzyme is blocked, where the hydrophobic and anionic affinity between the amino acids residues on both the bioactive peptides and the enzyme causes the occupation of its

active site **(Scaloni *et al.*, 1992 ; Akbarian *et al.*, 2022 ; Fu *et* Nguyen, 2022 ; Nong *et* Hsu, 2022).**

Nevertheless, the DUH inhibition ability could be due to the presence of molecules creating a covalent bind with ser200 (amino acid) of the enzyme active site **(Colletier *et al.*, 2006)**. Eventually, that leads to a competitive inhibition preventing the Ach binding with the AChE. Additionally, blocking the enzyme activity by the tested samples can be caused by the fixation of positive charged compounds on the anion subside which is the choline binding pocked of the enzyme catalytic site **(Agatonovic-Kustrin *et al.*, 2018)**.

Furthermore, both DM and DU's constituents demonstrated a notable mobility and ionic content as represented by the zeta-analysis. This mobility increases their possible contact with the enzyme active site **(Yabukarski *et al.*, 2020 ; Yang *et* Bahar, 2005)**. Though, the founded phenolic compounds can also participate in the inhibition of the AChE enzyme. In point of fact, it has cited in multiple researches that phenols have a great ability to block the AChE activity **(Dizdar *et al.*, 2018 ; Jabir *et al.*, 2018 ; Gasca *et al.*, 2020).** However, the small detected amount of those compounds doesn't explain the total inhibitory capacity revealed by all the samples.

Forming new bounds between each liquid hydrolysates molecules reducing their availability to link with the active site amino acids is the possible reason of the low inhibition capacity demonstrated by the two mixtures. Nevertheless, the low electrophoretic mobility of the mixtures' compounds and their ionization stage, minimize their ability to react with the enzyme domain **(Ouimet *et al.*, 2017, Abou-Diab *et al.*, 2021)**.

The deficiency of Ach neurotransmitter due to the hyper activity of AChE enzyme is the principal cause of the Alzheimer disease (AD) **(Ferreira-Vieira *et al.*, 2016 ; Stanciu *et al.*, 2019)**. Therefore, from the obtained data, the dromedary milk and urine contain neuroprotective molecules activity.

Noting that the DM, DU and their mixtures AChE inhibition capacity was never been investigated by previous researches. Furthermore, molecular interactions, low mobility and small content in peptides may be the reason of the low activity to block the AChE functionality by the DMUH1 and DMUH2 comparing to the DMH and DUH.

III.3.2. Antioxidant capacity

Both the scavenging capacity against the ABTS radical and the ferric reducing ability of the samples' hydrolysates are illustrated in figure 27.

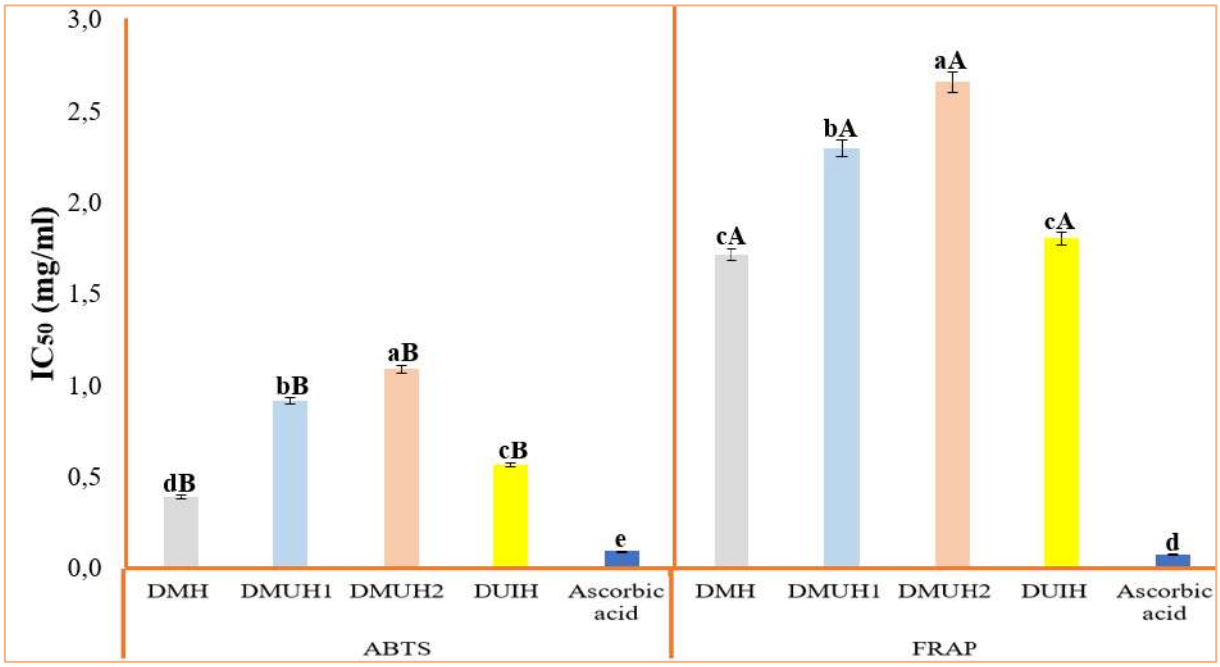

Figure 27. Hydrolysates IC_{50} obtained from the ABTS and FRAP assays

a-c: Different letters in the column indicate a significant difference (P <0.05) between the results of same camel samples; A-C: Different letters in the line indicate a significant difference (P <0.05) between the results of different

The substances released from the *in vitro* digestion of the four samples showed the ability to reduce the ferric ion (Fe^{3+}) into ferrous ion (Fe^{2+}) and to trap the ABTS free radical. However, the hydrolysates were more active in the scavenging test than the FRAP assay (Figure 27).

The DMH was the most active sample in both assays comparing to the three others hydrolysates, where it documented the lower IC_{50} of 0.385±0.085mg/ml and 1.710±0.237mg/ml in the ABTS and FRAP testes, respectively. The DUH also recorded an important inhibition against the ABTS radical with an IC_{50} of 0.559±0.017mg/ml and a remarkable reducing power activity in FRAP assay that showed a IC_{50} value of 1.797±0.183mg/ml (Figure 27).

The two mixtures hydrolysates demonstrated the lowest activities with the highest IC_{50} in the two tests. Despite the convergence in the IC_{50} values of the DMUH1 and DMUH2, yet

the DMUH1 is considered to be more active than the DMUH2 which registrated an IC_{50} bigger than the one recorded by the first formula (Figure 27).

The ascorbic acid used as the positive control in both assays presented the lowest IC_{50} reflecting the most important antioxidant activity comparing to the four hydrolysates (Figure 27).

The antioxidant ability of DMH in both tests can be regarded to the presence of the bioactive peptides and amino acids released from *in vitro* digestion of milk proteins **(Chi *et al.*, 2015 ; Soleymanzadeh *et al.*, 2016 ; Homayouni-Tabrizi *et al.*, 2017 ; Ibrahim *et al.*, 2018 ; Khan *et al.*, 2021)**. Furthermore, the milk hydrolysates antioxidant capacity could be resulted by the existence of phenolic compounds which were detected by HPLC analyses in the DMH **(Bouchouka *et al.*, 2012 ; Cicerale *et al.*, 2010 ; Minatel *et al.*, 2017 ; Li *et al.*, 2021)**.

The reducing capacity expressed by DUH may be related to the presence of molecules containing the OH functional group **(Al-Mamary *et* Moussa, 2021)**. Indeed, the FTIR scanning of the DUH documented a high absorbance, reflecting the OH group, which was proven to reduce and trap the free radicals and irons **(Al-Mamary *et* Moussa, 2021 ; Gülcin, 2012 ; Priyadarsini *et al.*, 2003 ; Silva et al., 2002)**. Moreover, some phenolic elements which may have participated in the antioxidant activity showed by these hydrolysates were also revealed by the HPLC analysis **(Bouchouka *et al.*, 2012 ; Cicerale *et al.*, 2010 ; Minatel *et al.*, 2017 ; Li *et al.*, 2021)**.

The activity expressed by both mixtures can be easily explained by the interaction and the new bounding formation among the active molecules. Precisely, between the functional groups of each liquid which led to the decreasing of those formulas' hydrolysates activity **(Abdelsattar *et al.*, 2021)**.

Oxidative stress can cause multiple health disorders such as AD disease and diabetic **(Markesbery, 1999 ; Reddy *et al.*, 2009 ; Chen *et al.*, 2012)**. Thus, the more the oxidation is inhibited and free radicals are trapped, the less the body is susceptible to those health problems, especially neural cells deterioration which lead to neurodegeneration problems and hyperglycemia pathologies **(Lobo *et al.*, 2010 ; de Oliveira *et al.*, 2012 ; Shankar *et* Mehendale, 2014 ; Phaniendra *et al.*, 2015)**. Therefore, the consumption of the four liquids in general can apport to the human body substances with an antioxidant capacity.

The bioactive substances with radical scavenging capacity and reducing power in DMH and DUH could be engaged in new bounds formation decreasing the two mixtures antioxidant capacity.

III.3.3. Antidiabetic activity

The IC_{50} of the DMH, DMUH, DMUH1 and DMUH2 against α-amylase and α-glucosidase is represented in figure 28.

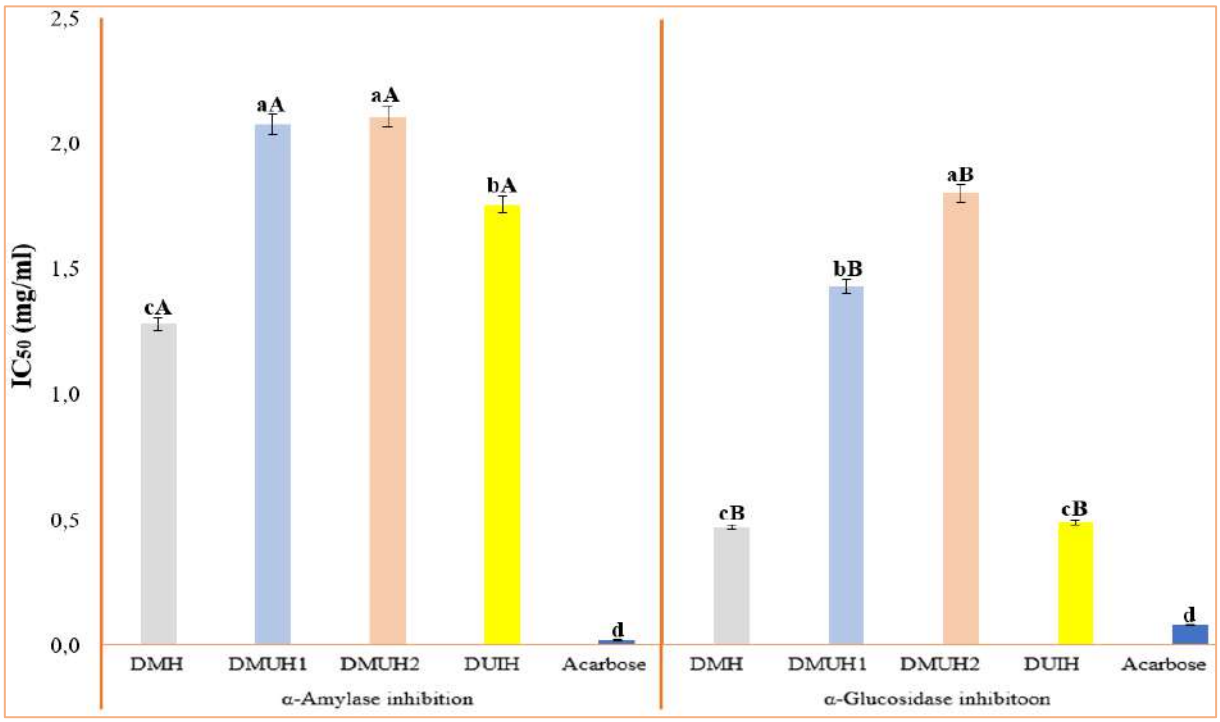

Figure 28. Hydrolysates IC_{50} against α-amylase and α-glucosidase activities

a-c: Different letters in the column indicate a significant difference (P <0.05) between the results of same camel samples; A-C: Different letters in the line indicate a significant difference (P <0.05) between the results of different

A remarkable capacity to inhibit the α-amylase and α-glucosidase activities was documented by the four digested samples. However, DMH and DUH were more active separately then combined. Indeed, the two mixtures hydrolysates showed the higher IC_{50} reflecting a weak activity in both inhibition assays (Figure 28).

The digested sample with the highest ability to stop the α-amylase activity was the DMH that presented an IC_{50} of 1.280±0.201mg/ml, followed by the DUH that registrated a value of 1.757±0.189mg/ml as IC_{50}, while the DMUH1 showed an IC_{50} of 2.076±0.457mg/ml. The weakest capacity to inhibit this enzyme's functionality was represented by the DMUH2 that recorded an IC_{50} of 2.106±0.435mg/ml (Figure 28).

The ability of hydrolysates to block α-glucosidase activity was in the same order than in the α-amylase assay. Both milk and urine digested samples demonstrated a same inhibition capacity level against this enzyme activity. However, the highest ability to block the α-glucosidase functionality was showed by the DMH that exhibited the lowest IC_{50} of 0.470±0.099mg/ml, while the DUH showed an IC_{50} of 0.488±0.097mg/ml. The lower ability to inactive this enzyme was represented by the two formulas hydrolysates, where the DMUH1 recorded an IC_{50} three times higher than both liquids separately (1.429±0.324mg/ml) and the DMUH2 presented an IC_{50} almost four times higher with a value of 1.802±0.493mg/ml (Figure 28).

The higher activity against the two enzymes functionality was documented by the acarbose as a positive control, which registrated the minor IC_{50} of 0.021±0.001mg/ml and 0.081±0.002mg/ml in the α-amylase and α-glucosidase inhibition tests, respectively (Figure 28).

The ability to inhibit the α-amylase and α-glucosidase by DMH could mostly be related to the presence of bio-active peptides and amino acids generated from the *in vitro* digestion of the DM's proteins. Those peptides can create a covalent and temporary linkages with the catalytic domain's amino acids as the Asp of the superior side or the glutamate of the alpha-amylase enzyme **(Awosika *et al.*, 2019 ; Baba *et al.*, 2021 ; Gómez-Villegas *et al.*, 2021 ; Gammoh et *al.*, 2022)**.

Moreover, the DMH FTIR profile revealed the existence of the OH and the CO groups that have the possibility to create a covalent bonding with both amino acids of the active site having a negative charge **(Albanese, 2012 ; Choudhary *et* Mishra, 2017 ; Alhakamy *et al.*, 2022)**. Indeed, blocking the positive carbon of the starch's glucose units from binding with the enzyme's active site, deceases its ability to break the saccharides bounds **(Hidayatul *et* Gan 2016 ; Soriano-García *et* Victoria 2018, Esfandi *et al.*, 2022)**.

Besides the ability to inhibit alpha-amylase, DMH bioactive peptides and amino acids demonstrated a remarkable capacity to minimize the α- glucosidase activity, epically the one generated by the degradation of the whey's protein as was reported in previous studies **(Kamal *et al.*, 2018 ; Baba *et al.*, 2021)**.

The obtained results confirm that DMH has an important role to minimize the digestion processes resulting in free carbohydrates by blocking both α-amylase and α-glucosidase activities, which are the two major key enzymes liberating glucose that moves

across the intestines membrane to the bloodstream **(Chegeni, 2014 ; Tundis *et al.*, 2010 ; Garcia-Naveiro *et* Udall, 2011 ; Alqahtani *et al.*, 2019)**.

The DUH activity would be due to the presence of excreted molecules by the *Camelus dromedarius* kidneys that can create bounds with the amino acids' active site of each enzyme, inhibiting by that the formation of the enzyme-substrate complex **(Tundis *et al.*, 2010 ; Garcia-Naveiro *et* Udall, 2011 ; Alqahtani *et al.*, 2019 ; Gómez-Villegas *et al.*, 2021 ; Gammoh et *al.*, 2022**).

Furthermore, the capacity of DUH to decrease the α-amylase functionality could be caused by the binding of the DUH molecules with the α-amylase's A domain catalytic site that consists mainly of three active amino acids Asp231, Glu 261 and Asp 185 **(Nielsen *et al.*, 1999 ; Brayer *et al.*, 2000 ; Lowe, 2004 ; Mehta *et* Satyanarayana, 2016)**. This mechanism is based on presence of a proton donor for the deprotonated Asp 231. Indeed, the DUH contains molecules with an hydroxyl group (FTIR profile) preventing the fixation of the substrate (starch) on the active site **(Nielsen *et al.*, 1999 ; Aghajari *et al.*, 2002 ; Taniguchi *et* Honnda, 2009 ; Ju *et al.*, 2019)**.

Moreover, this functional group also have the ability to bind with the catalytic domain of the α-glycosidase that consist of more than 15 amino acids participating in hydrolysis process, increasing the binding possibilities between the DUH molecules and the enzyme catalytic domain **(Iwanami *et al.*, 1995 ; Kimura *et al.*, 1997 ; Sugawara *et al.*, 2009 ; Amin *et al.*, 2019 ; Huang *et al.*, 2019 ; He *et al.*, 2022)**. That's why the inhibition capacity demonstrated by the DUH samples against this enzyme was higher compared to the α-amylase. Therefore, minimizing the functionality of the carbohydrate-digesting enzymes by DUH produces a postprandial antihyperglycemic effect by reducing the rate and extent of glucose uptake **(Hanhineva *et al.*, 2010 ; Oboh *et al.*, 2014 ; Blanco *et* Blanco, 2017 ; Pasmans *et al.*, 2022)**.

The drop down in the ability of both DM and DU after being combined would be due to the molecular interaction of the active compounds of each liquid. Indeed, creating new bounds between each hydrolysates functional groups will eliminate their bounding possibility with the amino acids of the enzymes' active domains. Nevertheless, the low molecular movement and ionizability and peptides concentration in DMUH1 and DMUH2 can also be the reason of minimizing their ability to block the enzymes activity.

Despite the very important antidiabetic activity of both dromedary milk and urine, the two mixtures recorded a lower postprandial antihyperglycemic ability. In fact, the more urine is added to the milk, the more interaction between the active molecules is possible. The less the two formulas are able to inhibit the two enzymes' activities, the less the antidiabetic capacity is exhibit.

II.3.4. Pancreatic lipase inhibition capacity

The capacities of the hydrolysates samples to inhibit the pancreatic lipase activity reflected by the IC_{50} values are demonstrated in figure 29.

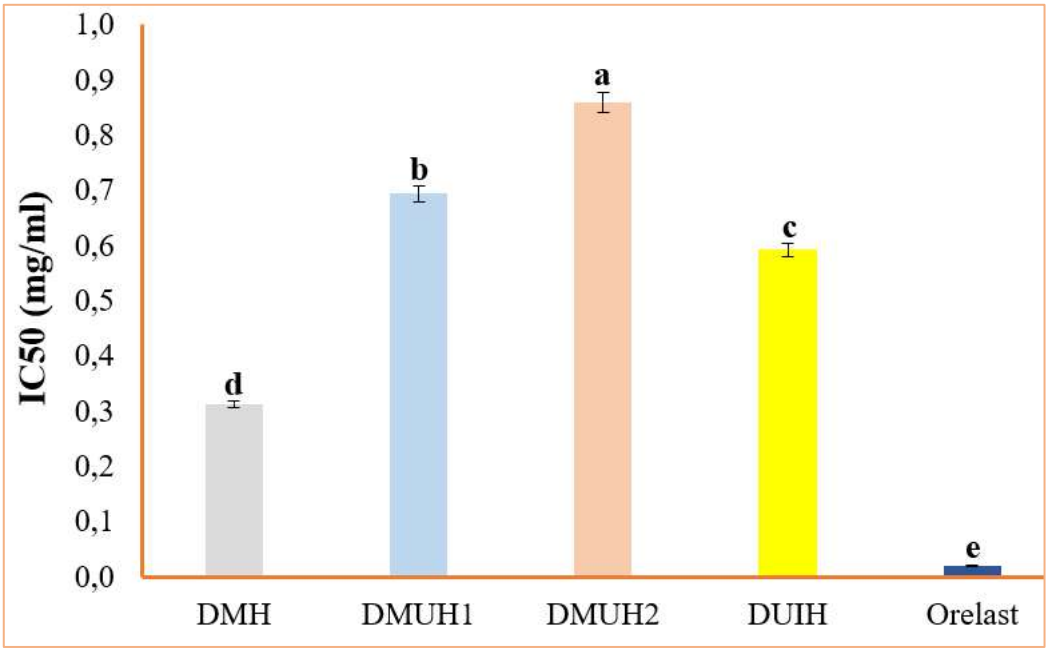

Figure 29. Hydrolysates IC_{50} obtained from the pancreatic lipase inhibition test

a-c: Different letters in the column indicate a significant difference ($P < 0.05$) between the results of same camel samples

The four hydrolysates' molecules revealed a capacity to stop the pancreatic lipase activity. The highest ability was recorded by the DMH that presented the lowest IC_{50} which was more than 1.8 times minor than the one registrated by the DUH. The activity of the two mixtures was lower than both liquids separately, where the DMUH1 registrated an IC_{50} of 0.694±0.071mg/ml, while the DMUH2 showed a value of 0.859±0.09mg/ml as the IC_{50} (Figure 29).

The orelast which was the positive control in this test was the most active compound comparing to the four hydrolysates, where it recorded the lowest IC_{50} which was 0.02±0.002mg/ml (Figure 29).

The DMH ability to inhibit the lipase activity may be caused by blocking the lid site of its catalytic domain by activating the Ser hydroxyl group of the active site, decreasing its nucleophilicity, which prevents the attaching between the substrate carbonyl group and the Ser **(Lowe, 1992 ; Colin *et al.*, 2008 ; Jafar *et al.*, 2018 ; Kamal *et al.*, 2018 ; Mudgil *et al.*, 2018 ; Baba *et al.*, 2021)**. Though, the DMH substance mostly peptides can form a links with the His and Asp of the active domain inhibiting the forming of any bound between the enzyme and its substrate, reducing by that the degradation of triglycerides and the liberating of glycerol and fatty acids **(Lands, 1965 ; Lowe, 1992 ; Langin, 2006 ; Feuerstein *et al.*, 2007 ; Jafar *et al.*, 2018)**.

The DUH inhibition activity can be explained by the presence of the "OH" group as shown in the FTIR spectrum. This group acts as hydrogen donor to the His of the catalytic region, inhibiting the deprotonation of the Ser **(Lüthi-Peng *et al.*, 1992 ; Takwa, 2010 ; Brockman, 2013 ; Khan *et al.*, 2017 ; Park *et* Park, 2022)**.

Moreover, this activity may also be due to the presence of molecules with a carbonyl group that bind directly with catalytic domain's Ser, inhibiting the carbonyl group fixation of the triacylglycerol **(Lüthi-Peng *et al.*, 1992 ; Hui *et* Howles, 2002 ; Brockman, 2013 ; Cen *et al.*, 2019 ; Zhao *et al.*, 2022)**.

Nevertheless, the important mobility of the compounds existing in the DMH and DUH can have a great participation in the activity demonstrated by both samples. In fact, the more substances are moving, the more their interaction with the amino acids active site is higher **(Alberi *et al.*, 1983 ; Yang *et* Bahar, 2005 ; Weng *et al.*, 2011 ; Darby *et al.*, 2017)**.

As the previous tests, the activity of both DMH and DUH's molecules was reduced after combination due to their involvement in new bound. In fact, despite the existence of peptides revealed by the amid groups and the OH group in their constituent yet both formulas couldn't reach the capacity demonstrated by the two liquids separately. However, the four samples manage to inhibit the pancreatic lipase hydrolysis process, results in stimulating weight loss and preventing weight gain, either way minimizing the risk of developing obesity and T2D **(Heck *et al.*, 2000 ; Han *et al.*, 2001 ; Melnikova *et* Wages, 2006 ; Kim *et al.*, 2016 ; Liu *et al.*, 2020)**.

The biological reaction between the DMH and DUH's active compounds decreases their ability to inhibit the pancreatic lipase activity as demonstrated by the

DMUH1 and DMUH2 IC_{50}. Moreover, all hydrolysates developed an antiobesity activity.

III.3.5. Statistical analysis

Using the Excel software, the PCA analysis related to the therapeutical activities of the four hydrolysates are illustrated in figure 30.

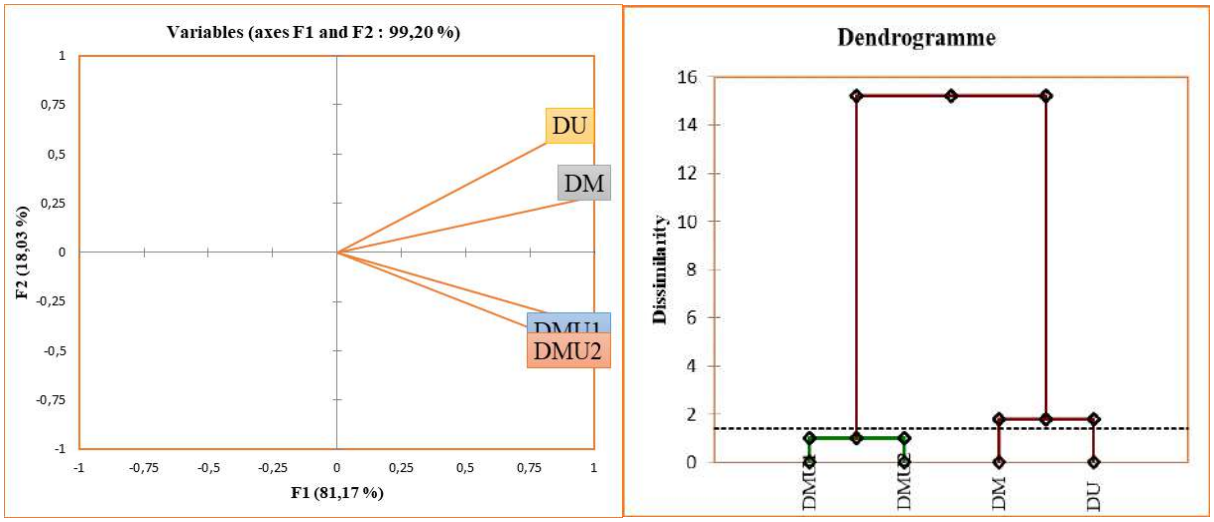

Figure 30. Principal component analysis (PCA) of variables and observation results

Basing on the IC_{50} values obtained by the digest samples in the six activities, the statistical analysis of the four different samples revealed a very important differences between the two digested liquids alone and combined. Where, the difference between the two axes variable F1 and F2 (91.80%) was higher than 80% (Figure 30).

Despite the large difference between the milk and urine samples, the variance and biplot analysis showed a similarity between the DMH and DUH in the six assays. The two formulas hydrolysates also presented a close IC_{50} in all tests, explaining their statistical rapprochement (Figure 30).

Nevertheless, the results obtained from the final analysis revealed in the above dendrogram (Figure 30), that the four digested samples are divided in two classes. The first one composes from the DUH and DMH, while the second class contains the two mixture hydrolysates. However, each class is divided in two sub class containing each liquid alone (Figure 30).

The statistical analysis showed the rapprochement of the milk and urine digested samples in which they were set in the same group constructed on the IC_{50} values, while the two formulas hydrolysates was classed in a separate group distinct from both DMH and DUH.

Despite the age, lactation stage and the food intake difference between the dromedary camels, all milk, urine and the two mixtures hydrolysates showed an important activity in the selected tests. However, some significant differences were noted between the same product hydrolysates in the same tests.

It was found that mixing dromedary milk with dromedary urine doesn't increase their individual capacities (in the selected tests). Indeed, the incorporation of urine to the DM can negatively affect DM protein's structure and conformation, decreasing and modifying by that their digestibility by the pepsin and trypsin.

As was pointed in the characterization part, modifying the milk protein environment such as the pH augmentation and the increasing of the mineral content can enhance their potentiality and functionality led to a protein oxidation (breaking the SS bounds), influencing by that the activity of the released peptides after hydrolysis process. Therefore, the DMUH1 and DMUH2 demonstrated a low activity comparing to DMH and DUH.

Moreover, it has been documented by the TSS characterization and FTIR analysis the possible presence of a new linkages between the two liquids constituents. Indeed, even the analysis of the hydrolysates' samples after the *in vitro* digestion by the FTIR revealed a clear existence of a molecular interactions between the both liquids compounds. Indeed, the same analysis revealed a clear existence of a molecular interactions between the two liquids compounds, especially the amidic bounds that are essentially linked to the milk peptides.

Nevertheless, the SDS-PAGE electrophoresis of the released peptides demonstrated that both formulas contain less peptides than the DM. In fact, the less bioactive peptides are in the sample, the less activity is illustrated against the four enzymes. Pointing that the activity of the three-containing milk hydrolysates may be essentially related to the peptides. Nevertheless, the bounding between the milk active molecules with the urine's substances reduces their possibility of linking with the amino acids of the catalytic domain of each tested enzyme.

Besides, the addition of the urine to the milk, decreases the mobility and ionizability of the released substances after the *in vitro* hydrolysis possess. The less movement the molecules have, the more their contact with enzymes active site, free radicals and ions (ferric ion) are minimized.

Despite the existence of the OH group, some phenolic compounds, free peptides, amino acids and carbonyl groups, but the two formulas showed in all selected tests a miner activity comparing to the dromedary milk and urine separately, highlighting the presence of antagonist effect between their molecules, which decreases the inhibitory capacity against the α-amylase, α-glucosidase, acetylcholine esterase, pancreatic lipase activities and antioxidant capacity tests by the DMUH1 and DMUH2.

Conclusion et perspectives

Conclusion et perspectives

Les bienfaits offerts par le lait et l'urine du dromadaire sont traduit par leur large utilisation dans le traitement de plusieurs pathologies dans les régions sahariennes. En absence d'une formulation précise à base de ces deux produits biologiques, nous nous sommes proposés d'entreprendre la présente étude.

Les expérimentations réalisées lors de notre étude sont effectuées au niveau du laboratoire de Protection des Ecosystèmes en Zones Arides et Semi-Arides (ECOSYS), Faculté des Sciences de la Nature et de la Vie, Département des Sciences Biologiques de l'université Kasdi Merbah-Ouargla, aux laboratoires de Chimie et de Métallurgie du Département d'ingénierie alimentaire, Faculté de Chimie de l'Université de Yildiz Technical (Istanbul, Turquie) et au Centre de Recherche Scientifique et Technique en Analyses Physico- Chimiques (C.R.A.P.C) de Ouargla.

La caractérisation physico-chimique des liquides analysés révèle que le pH du DM est légèrement acide et celui du DU est légèrement basique. Les deux formules à base de DM et DU présentent un pH neutre qui peut affecter le processus d'ionisation, de mobilité, de conformation et d'hydrolyse des protéines du DM.

Les faibles teneurs des deux mélanges en TSS comparées à celles des deux liquides séparés suggèrent la formation de nouvelles liaisons entre les composés du DM et ceux de DU.

Les propriétés optiques de DMU1 et de DMU2 sont plus comparables à celles de DM que celles du DU. De plus, les paramètres de couleur obtenus sont en accord total avec l'évaluation à l'œil nu.

La disproportionnalité des concentrations des protéines et de molécules amides trouvées entre les deux mélanges, DM et DU en utilisant la technique FTIR, révèle une éventuelle implication ou la création de nouvelles liaisons amidiques entre les deux constituants de DM et DU après avoir mélangé, ce qui peut affecter les capacités protéiques du lait de dromadaire et leur fonctionnalité.

La teneur en glucides du DMU1 et du DMU2 correspond à la quantité enregistrée dans les deux liquides séparés, éliminant la possibilité des interactions moléculaires entre les éléments glucidiques des molécules de DM et DU.

Une quantité lipidique proportionnelle est documentée dans les deux mélanges par rapport à la teneur enregistrée par le DM, supprimant la possibilité d'un engagement des constituent lipidiques du lait avec celles de l'urine.

Une augmentation remarquable de la quantité de calcium, sodium, potassium et chlorure est enregistrée dans les DMU1 et DMU2 dû à l'ajout de DU qu'a enregistré la teneur la plus élevée de ces minéraux par rapport au DM, ce qui peut influencer négativement la structure et la solubilité des protéines du lait.

La migration par électrophorèse montre l'existence d'un profil similaire avec neuf bandes de protéines dans le DM, DMU1 et DMU2 concernant les protéines du lait, ce qui minimise la possibilité de fragmentation des protéines. En outre, l'analyse SDS-PAGE confirme l'absence de protéines de poids moléculaire supérieur à 14KD dans les urines.

L'ajout d'urine provoque l'oxydation des protéines du DM en rompant leurs liaisons thiol. Une modification structurelle peut influencer la fonctionnalité et le processus de digestion de ces protéines.

L'analyse en composantes principales (ACP) basée sur l'analyse physico-chimique et biochimique divise les quatre échantillons testés en trois grandes classes mettant le DM et le DMU1 dans la même classe, reflétant leur grande proximité compositionnelle, le DMU2 dans un groupe séparé loin des deux échantillons et le DU qui est classé individuellement en raison de ses propriétés incomparable aux trois autres échantillons.

Il nous a été donné de constater après la digestion *in vitro* des DM, DU, DMU1 et DMU2 par des enzymes peptiques mise en évidence par la technique de SDS-PAGE, que les molécules de DU ne provoquent pas d'inhibition des activités de la pepsine et de la trypsine.

Le profil des groupes fonctionnels des hydrolysats des quatre échantillons obtenus par la technique FTIR montre l'existence des interactions moléculaires entre les substances DMH et DUH.

Par ailleurs, la détection quantitative et qualitative des composés phénoliques dans les hydrolysats en utilisant l'HPLC-DAD, révèle que la concentration de ces éléments dans les deux formules hydrolysats n'est pas exactement proportionnelle aux

composés détectés dans les deux liquides séparés. Ce constat peut être due à l'association des phénols à d'autres molécules. Néanmoins, la faible quantité de ces composés détectée dans les quatre hydrolysats éloigne la possibilité de leur participation aux activités des hydrolysats.

Le potentiel zêta et la mobilité détectés à l'aide du Zetasizer révèle le stade électrostatique du DMH, DUH et des deux hydrolysats qui ont montré une forte sensibilité à la coagulation, minimisant la mobilité électrophorétique de leur constituant contrairement à celle exprimée par les substances DMH et DUH.

La dernière partie de la thèse consacrée à l'évaluation de certaines activités thérapeutiques des quatre hydrolysats dont la capacité neuroprotectrice n'ayant pas fait l'objet de recherches antérieures. Les peptides libérés par la digestion *in vitro* des protéines du lait ont montré la plus grande capacité à inhiber l'activité AchE. C'est aussi le cas des molécules d'urine. Cette capacité est moins prononcée chez les deux mélanges issus de ces produits, ce qui peut être expliqué par leur faible teneur en peptides, les interactions moléculaires entre les deux liquides biologiques ainsi que la faible mobilité électrophorétique de leurs constituants.

En outre, une variation significative est documentée dans l'activité antioxydante par les hydrolats ; où l'IC_{50} du DMH obtenu par le test ABTS est quatre fois inférieure à l'IC_{50} exprimée dans le test FRAP. Par ailleurs, les échantillons d'urine sont plus actifs contre le radical ABTS que dans le test FRAP, ce qui a conduit à une différence notable dans l'activité antioxydante des hydrolysats des deux mélanges qui ont montrés l'activité la plus faible. La diminution de la capacité des deux liquides après leur réunion pourrait être due à des engagements des substances bioactives de DMH et DUH d en formant des nouvelles liaisons moléculaires.

Par ailleurs, l'activité antidiabétique des hydrolysats est obtenue en évaluant leurs capacités inhibitrices vis-à-vis de l'α-amylase et de l'α-glucosidase. Une grande variation de l'IC_{50} de tous les échantillons est démontrée entre les deux tests, dans lesquels l'activité la plus élevée est enregistrée par le DMH et le DUH. Par ailleurs, les deux échantillons DMUH1 et DMUH2 montrent une diminution remarquable dans les deux tests par rapport aux deux liquides digérés, abaissement possiblement causé par les liaisons des groupements fonctionnels et actifs entre les hydrolysats de DM et DU

et à la faible concentration en peptides, ainsi que le mouvement limité des molécules des deux mélanges.

L'inhibition de la lipase pancréatique est le test choisi pour la détermination de l'activité anti-obésité. Le DMH et le DUH montrent l'habilité la plus élevée à bloquer cet enzyme contrairement au DMUH1 et au DMUH2 qui ont montré une capacité plus faible. Cela peut être expliqué par l'association entre les composés actifs du lait et de l'urine, diminuant leur capacité à créer une liaison covalente avec le site actif de la lipase pancréatique.

L'analyse statistique (ACP) a classé les hydrolysats du lait et de l'urine dans le même groupe en fonction de leurs activités dans les tests sélectionnés (par rapport aux valeurs des IC_{50} obtenues). De l'autre côté, les deux hydrolysats DMUH1 et DMUH2 sont placés loin du DMH et du DUH dans la même classe en raison de leur IC_{50} exprimée dans les six activités testées.

De manière globale, les échantillons de lait et d'urine sont plus actifs séparément que combinés. En fait, plus on ajoute d'urine au lait, moins le mélange est actif. Par conséquent, le DMUH1 révèle une activité plus élevée que le DMUH2 dans les tests choisis. Une diminution de l'activité d'inhibition des enzymes sélectionnées et de la capacité antioxydante peut être principalement due au changement des conditions générales des protéines du lait comme indiqué dans la partie de caractérisation, ainsi au l'interférence moléculaire entre les substances actives de chaque liquide. Cela a finalement conduit à un effet antagoniste entre ces molécules réduisant leur capacité.

En conclusion, les quatre catégories d'échantillons montrent des activités très importantes dans les tests sélectionnés. De plus, le lait et l'urine de dromadaire semblaient à contenir des substances très actives résultant par les voies métaboliques de leur production par cet animal. En effet, quels que soient le stade de lactation, l'âge et le type d'alimentation des *Cumulus dromadaires*, son lait et son urine documentent des capacités neuroprotectrices, antidiabétiques, anti-obésité et antioxydantes non négligeable, mais mieux séparées que mélangées. C'est pour cela, il est recommandé de consommer chaque liquide seul plutôt que combiné, afin d'avoir de meilleurs résultats dans les activités mises en évidence.

Malgré l'importance des données documentées, les résultats obtenus restent partiels. Par conséquent, des travaux supplémentaires doivent être effectués consistant à :

- Mener une étude plus approfondie sur le lait de dromadaire pour déterminer les peptides et les acides aminés exacts responsables des activités trouvées.
- Réaliser des tests complémentaires sur la séparation des molécules existant dans l'urine de dromadaire d'intérêt biologique.
- Effectuez plus de caractérisation chimique et biochimique pour les deux liquides.
- Réaliser des travaux sur les interactions exactes et le mécanisme antagoniste entre les molécules actives du lait et de l'urine.
- De rechercher plus d'activités thérapeutiques que les deux liquides peuvent également offrir à la santé humaine indépendamment et combinés

Conclusion & perspectives

Conclusion and perspectives

Based on the common ideas about the benefits offered by dromedary milk and urine in the treatment of several pathological problems by the Saharan populations and in the absence of a precise formulation based on both biological products, this study was carried out.

The present work aimed to characterize the dromedary milk, urine and two formulations based on a mixture of both liquids at two different concentrations 9:1 (10%) and 8:2 (20%) v/v (DM: DU, respectively) on the physical, chemical and biochemical levels.

Furthermore, we also aimed to evaluate some therapeutical interests as the neuroprotective ability, antioxidant capacity, antidiabetic potential in addition to the antiobesity activity of both liquids separately and combined.

The experiments of our study were carried out at the Laboratory for the Protection of Ecosystems in Arid and Semi-Arid Zones (ECOSYS), Faculty of Nature and Life Sciences, Department of Biological Sciences of Kasdi Merbah University. Ouargla, at the Chemistry and Metallurgy laboratories of the Department of Food Engineering, Faculty of Chemistry of Yildiz Technical University (Istanbul, Turkey) and at the Center for Scientific and Technical Research in Physico-Chemical Analysis (C.R.A.P.C) of Ouargla.

The physico-chemical characterization revealed that DM's pH is a bit acid, while the DU's recorded slightly basic pH. The two formulas exhibited a neuter pH that can affect the DM's proteins ionization, mobility, conformation and hydrolysis process.

The two mixtures recorded a TSS content lower than the two liquids alone, highlighting the formation of new bounds between the DM and DU's compounds.

The optical proprieties of DMUH1 and DMUH2 were very close to the DM and far from those of the DU. Moreover, the obtained color parameters were in a total agreement with the naked eyes evaluation.

Due to the unproportionally proteins and amidic molecules' concentrations between the two mixtures, DM and DU using the FTIR technic, a possible involving or new amidic bounds creation between the two liquids constituent after being combined is revealed, which eventually affect the dromedary milk protein capacities and functionality.

The DMU1 and DMU2 carbohydrate content match with the amount recorded in both liquids separately, eliminating the possible molecular interactions including the sugar elements between DM and DU's molecules.

A proportional lipidic amount was documented in the two mixtures comparing to the DM's fats content, removing the possibility of milk fats engagement with the urine's ones.

A remarkable augmentation in the calcium, sodium, potassium and chloride amount was recorded in the DMU1 and DMU2, because of the DU addition that recorded the highest content of those electrolytes that can negatively influence milk proteins' structure and solubility.

The electrophoresis migration demonstrated the existence of very similar profile with nine proteins bands in the DM, DMU1 and DMU2 relating mostly to the milk's proteins, minimizing the possibility of proteins fragmentation. Moreover, the SDS-PAGE analysis confirmed the absence of protein with a molecular weight exceeding the 14KD in DU.

The addition of DU causes the oxidation of the DM's proteins by breaking their thiol linkages, a structural modification that can influence the functionality and digestion process of those proteins.

The principal component analysis (PCA) carried out on the physical, chemical and biochemical analysis divides the four tested samples in three major classes composing of the DM and DMU1 reflecting their high compositional closeness. The DMU2 in a separate group far from the two sample and the DU which was also categorized in an individual class due to its far proprieties comparing to other three samples.

The DM, DU, DMU1 and DMU2 were subjected to the *in vitro* digestion using peptic enzymes, that was confirmed by the SDS-PAGE analysis. Moreover, it was found that the DU's molecules doesn't cause any inhibition to the pepsin and trypsin activities.

The SDS-PAGE was also used in another gradient to detect the released peptides from the *in vitro* hydrolysis assay which showed the low content of those peptides in the two formulas comparing the DMH sample.

The four samples' hydrolysates functional groups were investigated by the FTIR technic that showed the existence of a possible molecular interactions between the DMH and DUH substances.

Moreover, the hydrolysates were also subjected to an HPLC-DAD analysis for the quantitative and qualitative detection of phenolic compounds, where the concentration of those elements in the two formulas hydrolysates wasn't exactly proportional to the detected compounds in both liquids alone. Unmatching that can be due to the bounding engagement of phenols with other molecules. Nevertheless, the detected amount of those compound in the four hydrolysates is very minor eliminating their major participation in the hydrolysates' detected capacities.

The zetapotential and mobility using the Zetasizer analysis revealed the electrostatic stage of the DMH, DMUH1, DMUH2 and DUH, highlighting the high coagulation susceptibility of the two formulas hydrolysates minimizing their electrophoretic mobility that was lower than the one expressed by both DMH and DUH substances.

The last part of the dissertation was devoted on investigating some therapeutical forces, including the neuroprotective capacity that has never been studied previously for the DMH, DUH and two mixtures hydrolysates, applying the AChE inhibition test. In which, the released peptides from milk proteins' *in vitro* digestion showed the highest ability to inhibit the AChE activity, in addition to the urine molecules. While the two mixtures presented the minor activity against this enzyme possibly explained by the content in peptides, molecular interactions and low electrophoretic mobility.

Moreover, a significance variation was documented in the antioxidant activity were the IC_{50} of the DMH in the ABTS teste was four times lower than the IC_{50} expressed in the FRAP assay. While, the urine samples were more active against the ABTS radical than the FRAP test which led to a notable difference in the antioxidant activity of both mixtures hydrolysates that recorded the lowest activity. The decreasing of both liquids ability after reunion could be due to the engagement of the bioactive substances with radical scavenging capacity and reducing power in DMH and DUH in new bounds formation or molecular interference.

Furthermore, the hydrolysates antidiabetic activity was carried out by evaluating their inhibition capacities versus α-amylase and α-glucosidase. A great variation in the IC_{50} of the all the samples was demonstrated between the two tests, in which the higher activity was recorded by the DMH and DUH. However, the active functional groups binding between each other of each liquid hydrolysates, the low peptides concentration and the slow movement could be the reason of the both formulas' hydrolysates activity diminution in both assays.

Nevertheless, the pancreatic lipase inhibition was the chosen test for the antiobesity activity determination. Both DMH and DUH showed the highest ability to block this enzyme activity contrary to the DMUH1 and DMUH2 that showed a lower capacity. This can be caused by the biological reaction and association between the milk and urine active compounds decreases their ability to create a covalent binding with the pancreatic lipase active site.

The statistical analysis (PCA) classified the milk and urine hydrolysates in the same group basing on their activities in the selective testes (IC_{50} results) despite their huge distinctions on the characteristics level. On the other side, the two mixture hydrolysates were placed distinct from both DMH and DUH in the same class because of the expressed IC_{50} reflecting the lower capacity in six assays.

In a global way, both milk and urine samples were more active separately than combined. In fact, the more urine is added the less the mixture is active. Therefore, the DMUH1 recorded a higher activity than DMUH2 in the chosen tests. A decreasing in the selected enzymes inhibition activity and the antioxidant capacity can mainly be due to the changing in the general milk proteins conditions as pointed in the characterization part, in addition to the molecular interference between the active substances of each liquid. This eventually led to an antagonist affect between those molecules reducing their ability.

Concluding that, the four samples' types showed very important activities in the selected tests. Moreover, both of dromedary milk and dromedary urine appeared to have a very actives substances resulting from the metabolic pathways of their production. Indeed, no matter is the lactation stage, age and the food intake by the *Cumulus dromedaries*, its milk and urine documented an underestimated neuroprotective, antidiabetic, antiobesity and antioxidant capacities yet separately better than mixed. Therefore, it is recommended to consume each liquid alone rather than combined, in order to have better results in the highlighted activities.

Despite the importance of the documented data, the obtained results remain partial. Therefore, some additional work needs to carry out consisting of:

- Conduct further study on dromedary milk to determine the exact peptides and amino acids responsible of the founded activities.
- Carrying supplementary researches on separating the molecules existing in dromedary urine with biological interest.
- Run more characterization on the chemical and biochemical levels for both liquids.

- Realize more work on the exact interaction and the antagonist mechanism between both milk and urine active molecules.
- To investigate more therapeutical activities that both liquids can also offer to the human health independently and combined.

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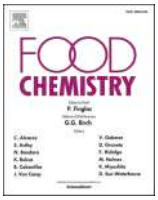

Physical and biochemical characterization of dromedary milk as traditionally consumed by Bedouins

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ABSTRACT

Few studies were dedicated to characterizing the dromedary milk (DM) as consumed by Bedouins, mixed with a small amount of dromedary urine (DU). Therefore, this work aimed to reveal some physical and biochemical characteristics of the DM alone, and incorporated with DU at two different concentrations. pH, colour, Brix, fats, and minerals were evaluated with proteins and sugar content using FTIR; in addition to protein oxidation. The results showed that DM counts as a rich source of the nutritional element yet its proprieties were affected by the addition of DU. An important increase in colour, sugar, fat, and protein concentration with a remarkable augmentation in the pH, mineral content, and protein oxidation where the more urine is added the more milk proteins are oxidated as demonstrated by the characteristics showed in the two mixtures which will negatively affect DM therapeutical proprieties, especially the one related to proteins.

1. Introduction

In recent years camel milk started to occupy an important place in the dairy section research on several levels due to its physical properties, chemical composition, functional characteristics, and its therapeutic utility (Ho, Zou, & Bansal, 2021). Dromedary milk is one of the most important rich foods due to its specific and balanced composition in all the nutrients proteins, fats, lactose, mineral salts, vitamins, and water (Hammam and Agriculture, 2019). The physiological, biological, and metabolic proprieties of the *Camelus dromedarius* influence the biosynthesis pathways of this biological fluid by producing a unique and highly homogenized liquid rich in molecules characterized by several therapeutic properties that have been confirmed by many scientific types of research (Ali et al., 2019).

For the Bedouins population of arid and semi-arid regions of the world in general and Algeria in specific camel milk is not always consumed alone, but mixed with a small amount of camel urine which is counted to be an effective solution and candidate in the alternative medicine for multiple health disorders for the population of these areas. So far, few important roles were revealed in camel urine such as the ability to stop the proliferation of some microbial strains with a great antibacterial activity which makes it microbial-free (Sumia & Majid, 2016). It can also inhibit the multiplication of some cancer cells *in-vitro*, additionally to some antiplatelet and antidiabetic proprieties (Salamt, Idrus, Kashim, & Mokhtar, 2021). However, despite its wide usage in the Saharan region, the expansion in ~~the~~ dromedary urine research remains limited due to the hard acceptance of the consumption of this urine as a therapeutic product in many parts of the world.

Up to now, several papers and works have been devoted to studying dromedary milk and its general proprieties on many levels, while no studies were dedicated to reveal the possible changes that can influence the physical and biochemical proprieties of the milk after being

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combined with dromedary urine despite its wide consumption and utilization as a healing product by the African Bedouins especially the Algerians who own more than 417 thousand of the *Camelus dromedarius* (Alkhamees et al., 2017; EL-Elyani and Khalifa, 2006). Therefore, the following work aimed to reveal changes that can accrue to the dromedary milk by evaluating some physical and chemical characteristics of both the dromedary milk and urine separately and combined at two different concentrations (DM: DU at 9:1 and 8:2 v/v).

2. Materials and method

2.1. Sampling

The used samples of milk and urine were obtained from ten female camels living in the arid extensive of Ouargla, Algeria. All animals were examined by a veterinary and were disease free, aged between 4 and 10 years under different lactation stages, and fed only on the Saharan plants with free access to wells and oases water. The samples were collected in the early morning (4–6 am) in sterile amber glass bottles and were immediately cooled and transported to the laboratory for further analysis that was accomplished on: Dromedary milk (DM), Dromedary urine (DU), DM incorporated DU with 9: 1 v: v (DMU1), and DM incorporated with DU with 8:2 v: v (DMU2), that was chosen dilutions were suggested after interrogation of nearly 60 people, and which combination do they prefer.

2.2. Physical and biochemical characterization

2.2.1. pH

From a solution consisting of 10 mL of the fresh sample, the pH was determined at room temperature with the use of a pH meter (Thermo-Scientific Orion Star A111, Indonesia) that was calibrated before each measurement with a standard buffer.

2.2.2. The total soluble solids content (Brix)

A suitable amount of the fresh sample was placed on the prism-plate of the Digital-Bench- Refractometer (model 10,430 with Porx reading range 0–30 Bausch and Lomb Co. California, USA) that was cleaned and calibrated before each measurement with distilled water, the rate of the soluble solids appearing on the device screen is recorded as Brix (Salih et al., 2019).

2.2.3. Optical properties (Color)

As described by Milovanovic et al. (2020) the color of the studied samples was evaluated. The three-color values L*, a* the b* were recorded with a colorimeter (Konica Minolta CR-400, Japan). (Milovanovic et al., 2020):

The L* indicator of whiteness-blackness varies from 0 (black) and 100 (white).

The a* designates redness-greenness with values that go from −60 (green) to + 60 (red).

The b* blueness-yellowness indicator confined between −60 (blue) and + 60 (yellow).

The device was cleaned and calibrated with the standard white plate values (L*= 93.87, a* = −0.55, and b* = 5.13) at room temperature and the measurements are carried out at 5 different points of each sample with three repetitions (n = 3).

2.3. Biochemical characterization

2.3.1. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE)

A volume of 20ul of a mixture prepared by mixing 200 μL of all the samples with 200 μL of the sample buffer and let boil for 5 min then left to cool down, was injected into the gel halls. Proteins separation was illustrated on gradient acrylamide gel of 7.5 %−20 % so all ranged proteins can be visualized, the separation was illustrated at a constant current of 40 mA/gel (Bio-Rad Laboratories, Inc., Richmond, CA, USA). The β-mercaptoethanol-bromophenol blue was used to dye the samples, and the electrophoresis gel was stained with the use of the Coomassie Brilliant Blue for 24 h and distained with methanol solution (50 %) for four hours (Muguruma et al., 2012).

The determination of the molecular weight of the proteins in interest was carried out by migrating in parallel (under the same conditions) with standard proteins of a known molecular weight.

2.3.2. Proteins and sugar quantification with FTIR

A volume of 10 μL of each sample was placed into the ATR crystal surface of a Bruker Tensor 27 spectrometer equipped with a DLA TGS detector (Bremen – Germany), at the mid-infrared area with a band interval of 4000–600 s^{-1} the spectrums were collected with a resolution of 4 cm^{-1} for the FTIR scanning recording (accumulation of 16scan/spectra). The data procuration was performed with the OPUS program (7.2 version for Windows from Bruker GMBH). The distilled water was used as a background to eliminate water bands absorbance and the samples traces were eliminated after each injection with deionized water and ethanol (80 % v: v).

The protein quantification was carried out by detecting the amide I band of the secondary structure of the protein that produces a peak in 1600 cm-1 1700 cm^{-1} area (Usoltsev, Sitnikova, Kajava, & Uspenskaya, 2019), the Bovine Serum Albumin (BSA, Sigma-Aldrich) protein was chosen for the calibration curve obtention with a concentration ranging from 1 mg/mL to 10 mg/mL (Fig. 1).

The sugar quantification was obtained by the absorbance spectra between 1180 and 960 cm^{-1}, precisely at 1035 cm^{-1}which corresponds to the glucose concentration (Nybacka, 2016), pure glucose (Sigma-Aldrich) was the reference for the sugar detection with a concentration goes from 1 mg/mL to 6 mg /mL (Fig. 2).

Each concentration from the used standards was scanned three times and the average of each concentration was taken for drawing the calibration curve. After each spectrum acquisition, ATR correction was performed (Figs. 1 and 2).

2.3.3. Fat content

The GERBER method was used to assess the fat content of the DM, DMU1, and DMU2. About 11 mL of milk samples are added to 10 mL of sulfuric acid (density = 1.5 g/mL) that acts on dissolving proteins and non-fat constituents. Next, isoamyl alcohol was added in small quantities (1 mL) to the butyrometer for the separation of fatty material. The sample, acid, and alcohol mixture were stirred to facilitate the dissolution of the various components of the milk and centrifuged for 10 min, the butyrometer was immersed vertically in a water bath brought to 70 °C for 5 min to promote the separation between the dissolved lipid phase and the other components.

The fat content (FC), was calculated using the following formula:

$$FC = B - A$$

where A and B are the readings taken at the lower and upper ends of the FC column, respectively.

2.3.4. Mineral's content

Following the procedure described by Zwierzchowski and Ametaj 2019, Calcium (Ca), Sodium (Na), Potassium (K), and chloride (Cl) were quantified using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). (Zwierzchowski & Ametaj, 2019).

2.3.4.1. Protein oxidation. A volume of 0.2 mL of each sample of the DM, DMU1, and DMU2 was introduced to 4 mL of Tris-EDTA buffer (pH 8), followed by the addition of 0.08 mL of DNTB solution (10 mM). After incubation for 15–20 min at room temperature, the mixture was centrifuged for 10 min at 3000g. The supernatant absorbance was

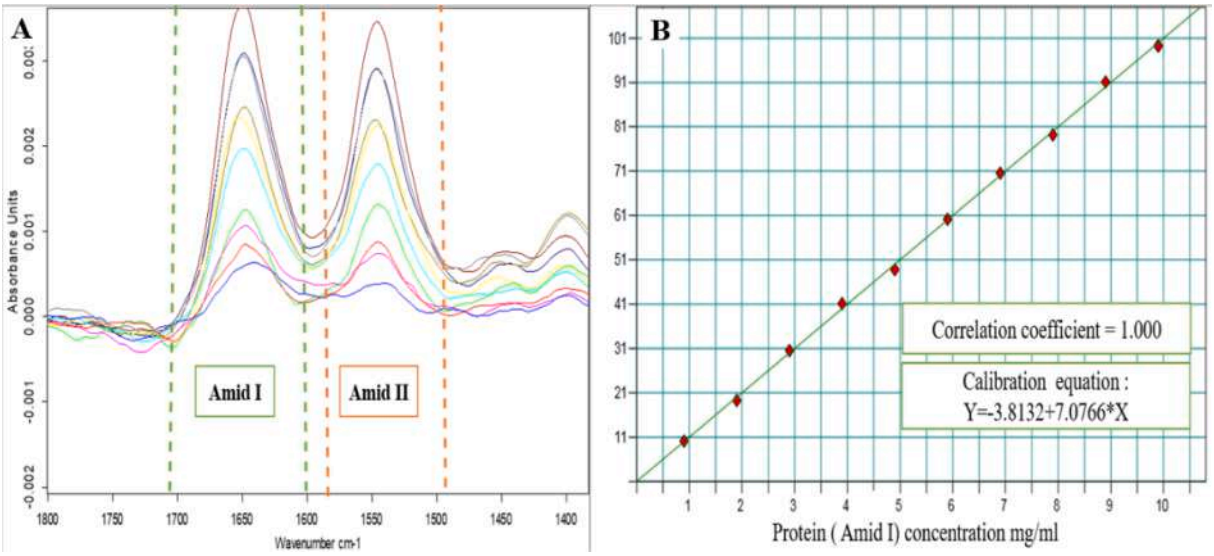

Fig. 1. A: The FTIR spectrum of the BSA standard protein in the Amid I with integration area of 1710.7 cm^{-1} – 1604.1 cm-1. **B:** Calibration curve of the amid I of the standard protein (BSA) obtained by the OPUS program (7.2 version for windows from BRUKER GMBH).

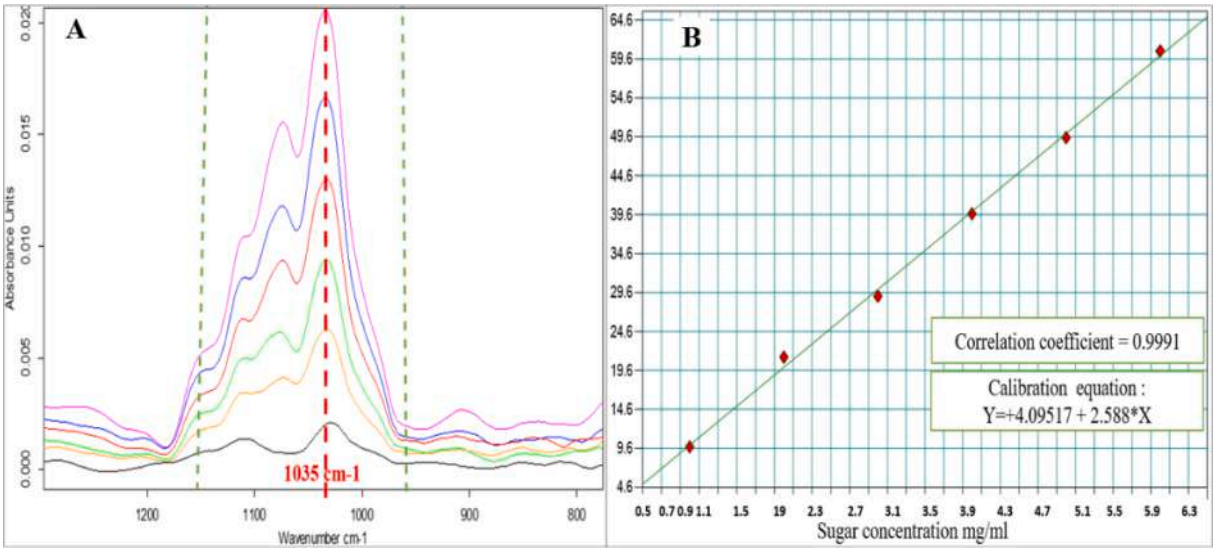

Fig. 2. A:The FTIR spectrum of sugar standard with integration area of 1180 and 960 cm-1, precisely at 1035 cm-1 that corresponds to the glucose concentration, B: Calibration curve of the sugar (Glucose) obtained by the OPUS program (7.2 version for windows from BRUKER GMBH),

recorded at 412 nm. Each sample was accompanied with a blank that was prepared in the same condition and in parallel with 0.03 mL of each sample and 4 mL of the buffer excluding the DNTB solution (Mahcene, 2021). The sulfhydryl groups (-SH) concentration was calculated with the following equation:

-SH (µmol/L) = (A – B – volume of the sample blank)*(solution volume/ volume of the sample)/ DNTB molar extinction coefficient.

A: sample absorbance.

B: blank absorbance.

2.4. Statistical analysis

Using the SPSS package program (IMB SPSS 25) Statistical analysis was carried out. Performing a One-way analysis of variance (ANOVA) and the statistical differences between the means were evaluated at the significance level of 0.05 using the Tukey test. The experimental analyses were performed in triplicate (n = 3).

3. Results and discussion

3.1. pH

The mean pH of the studies milk samples was 6.10 6 ± 0.68 with no-significant difference between the ten tested samples ($p < 0.05$) despite the lactation and age dissimilarity of the camels, the pH values that were recorded during the present study are within the range that varies from 5.7 to 6.7 as was reported by many authors who have worked on DM (Bouhaddaoui et al., 2019; Swelum et al., 2021), this slightly acid pH is most likely due to the high vitamin C content that was reported to be 24.9 mg /L (Bouhaddaoui, et al., 2019), it also depends on the casein and citrate content and could be affected by food and water availability(AL-Ayadhi and Halepoto, 2017). DU illustrated a high level (Alkaline) pH with a mean of 8.11 ± 0.41 ($p < 0.05$) almost similar to the value mentioned by (Anwar et al., 2021), which explain the documented increase of the pH level of the two mixtures DMU1 and DMU2 samples with values of 6.90 ± 0.25 and 7.15 ± 0.36, respectively.

The registered augmentation in the pH level of DMU1 and DMU2 can affect the milk compositions especially proteins and their potential functionality such as B-casein, and camel α-Lactalbumin. The change in DM pH means a change in the protein environment causing a modification in the protonation state of the amino acids atmos. Precisely, the swinging in the protein pH range affects the electrostatic interactions of the ionizable amino acids residues modifying by that the protein conformation, and structure which can manipulate its general functionality and solubility which also can negatively affect the digestion process of those proteins (Di Russo et al., 2012; Talley & Alexov, 2010).

3.2. The total soluble solids content (Brix)

A significant dissimilarity ($P > 0.05$) of the total content of soluble solids (Brix) was documented between the same sample category due to the variability of age and lactation state, in addition to food and water intake by the animals, and also between the DM, DMU1, DMU2, and DU that presented the highest average of the total soluble solids (TSS) amount with a value of 8,09 ± 0,014 (Table 1) a non-comparable value due the major lack of researches on DU characteristics, yet it counts as a high amount comparing to the human urines' TSS content, where dromedary kidneys illustrate an important ability to reabsorb water as a conservation path resulting the secretion of highly concentrated urine (Ali, Baby, & Vijayan, 2019).

As Table 1 illustrates, the DM samples presented a mean value of the TSS content of 6,89 ± 0,01 %, low compared to the average declared by previous researches on DM conducted in cooled weather (spring and winter) (Ayyash, Al-Nuaimi, Al-Mahadin, & Liu, 2018); The TSS content is related mainly to the weather (Seasonal) that controls water consumption by this animal with an inverse relation between TSS in DM and water consumption for the *Camelus dromedarius* (Raghvendar, 2017) causing a reduction of 4 % in the TSS in the summer (Haddadin, Gammoh, & Robinson, 2008) where the sampling was carried out.

The two mixtures illustrated the lowest TSS content with 6,06 ± 0,56 for the DMU1 and 6,654 ± 0,044 % for the DMU2 (Table 1), the recorded results were not proportional to the TSS in the two separate liquid milk and urine which can be explained by the interaction and new bounds formation between the molecules of milk and urines which cause decreasing in solids amount and their solubility (Tran & Tran, 2020).

3.3. Color optical properties (Color)

As Table 1 presents, all the DM samples present high values with a mean that reached 87 % of the L* value indicating a very bright color approaching white, contrary to the urine samples that showed an average of 17 % as an L* value with a color very far from white and tends more to the dark color explaining by that the noted decreasing in the brightness for both mixtures, where the more urine is added the less samples are white with a decreasing in the L* value recorded for the dMU1 and DMU2 (Table 2) yet higher than 79 % which make it included in the white zone. According to the litterateur, dromedary milk was always described with its very white opaque color compared to other milk samples (Swelum, et al., 2021) due to the fine homogenization and equivalent distribution of fats and casein micelles throughout the milk (Bakry et al., 2021).

No significant difference ($p < 0.05$) was revealed between the four types of samples whose values of a* obtained are negative reflecting by that a color close to green and far from red in the following order DM, DMU1, DMU2, and DU with the closest value to the green color (Table 1). All the samples showed an approximate value for this parameter leading to a convergence with the observation of the naked-eye.

The b* character covers the interval from yellow to blue, DM, DMU1, DMU2, and DU demonstrated a positive value for the mentioned character reflecting a color near to yellow, the highest value for the b* parameter was recorded by the DU sample with a value of 7,77 ± 0,38 that gradually decrease the more urine is reduced in the DMU1 and DMU2 (Table 1). Where the DMU1 reported a value of 3.36 ± 0.33 and the DMU2 5.69 ± 0.54 a significant augmentation ($p < 0.05$) that goes to the yellow color far from the milk color, in a total agreement with what appears to the viewer.

The obtained data are in perfect agreement with the visual evaluation of both liquids milk and urine, however, the unavailability of previous studies of the three-color parameters L*, a*, and b* put the obtained results in a non-comparable position for both DM and DU. The DMU1 and DMU2 seems to be closer to the pure DM because of the low doses of urine added and its good homogenization and integration with the milk compositions.

3.4. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE)

The SDS-PAGE of total protein of DM, DMU1, and DMU2 are illustrated in Fig. 3, the three samples containing milk exhibited almost identical profiles with a decrease in the color intensity of the protein bands the more amount of milk is eliminated reducing by that protein's concentration in DMU1 and DMU2, where no fragmentation or molecular denaturation was caused by the addition of the DU that shows a

Table 1
Physical properties of dromedary milk, urine, and their mixtures.

			DM	DMU1	DMU2	DU
pH		Min	$6.003 \pm 0.006^{(d)}$	$6.337 \pm 0.006^{(b)}$	$6.293 \pm 0.006^{(c)}$	$7.120 \pm 0.000^{(a)}$
		Max	$6.537 \pm 0.040^{(d)}$	$7.200 \pm 0.000^{(c)}$	$7.547 \pm 0.006^{(b)}$	$8.393 \pm 0.006^{(a)}$
		Mean	$6.269 \pm 0.059^{(d)}$	$6.898 \pm 0.006^{(c)}$	$7.153 \pm 0.001^{(b)}$	$8.133 \pm 0.003^{(a)}$
Brix %		Min	$6.133 \pm 0.115^{(a)}$	$5.767 \pm 0.058^{(b)}$	$5.333 \pm 0.115^{(c)}$	$4.133 \pm 0.115^{(d)}$
		Max	$8.000 \pm 0.000^{(d)}$	$8.200 \pm 0.000^{(c)}$	$8.867 \pm 0.115^{(b)}$	$12.000 \pm 0.000^{(a)}$
		Mean	$6.892 \pm 0.014^{(b)}$	$7.058 \pm 0.556^{(b)}$	$6.654 \pm 0.044^{(b)}$	$8.092 \pm 0/014^{(a)}$
Optical proprieties	a*	Min	$-1.690 \pm 0.017^{(a)}$	$-2.253 \pm 0.025^{(b)}$	$-3.090 \pm 0.046^{(d)}$	$-2.640 \pm 0.017^{(c)}$
		Max	$-0.893 \pm 0.015^{(a)}$	$-1.277 \pm 0.081^{(b)}$	$-1.840 \pm 0.020^{(c)}$	$-0.887 \pm 0.212^{(a)}$
		Mean	$-1.245 \pm 0.014^{(a)}$	$-1.765 \pm 0.025^{(b)}$	$-2.417 \pm 0.013^{(c)}$	$-1.713 \pm 0.049^{(b)}$
	b*	Min	$1.060 \pm 0.053^{(d)}$	$1.343 \pm 0.121^{(c)}$	$1.747 \pm 0.168^{(b)}$	$5.393 \pm 0.035^{(a)}$
		Max	$4.867 \pm 0.040^{(d)}$	$6.163 \pm 0.040^{(c)}$	$12.793 \pm 0.898^{(a)}$	$10.827 \pm 0.158^{(b)}$
		Mean	$2.491 \pm 0.078^{(c)}$	$3.305 \pm 0.328^{(c)}$	$5.689 \pm 0.541^{(b)}$	$7.769 \pm 0.379^{(a)}$
	L*	Min	$81.993 \pm 0{,}690^{(a)}$	$76.340 \pm 0.567^{(b)}$	$74.143 \pm 0.628^{(c)}$	$14.723 \pm 0.031^{(d)}$
		Max	$90.597 \pm 0.152^{(a)}$	$85.223 \pm 0.207^{(c)}$	$87.483 \pm 0.229^{(b)}$	$20.830 \pm 0.436^{(d)}$
		Mean	$86.970 \pm 0.107^{(a)}$	$80.852 \pm 1.241^{(b)}$	$79.818 \pm 1.274^{(b)}$	$17.233 \pm 0.164^{(c)}$

[a–d]: Different letters in the line indicate a significant difference ($P < 0.05$) between the results of different camel samples. DM: Dromedary milk, DMU1: Dromedary Milk-Urine mixture 9–1 (v/v), DMU2: Dromedary Milk-Urine mixture 8–2 (v/v), DU: Dromedary urine.

Table 2
Biochemical parameters of dromedary milk, urine, and their mixtures.

			DM	DMU1	DMU2	DU
Sugar (%)		Min	2.707 ± 0.090$^{(a)}$	2.658 ± 0.030$^{(a)}$	2.301 ± 0.080$^{(b)}$	1.271 ± 0.033$^{(c)}$
		Max	3.741 ± 0.021$^{(b)}$	3.681 ± 0.064$^{(b)}$	3.898 ± 0.046$^{(a)}$	2.641 ± 0.064$^{(c)}$
		Mean	3.353 ± 0.405$^{(a)}$	3.155 ± 0.376$^{(b)}$	2.987 ± 0.474$^{(b)}$	2.046 ± 0.456$^{(c)}$
Protein (%)		Min	2.441 ± 0.0116$^{(a)}$	2.236 ± 0.054$^{(b)}$	2.073 ± 0.049$^{(b)}$	1.243 ± 0.022$^{(c)}$
		Max	3.895 ± 0.025$^{(a)}$	3.669 ± 0.075$^{(b)}$	3.304 ± 0.075$^{(c)}$	2.274 ± 0.059$^{(d)}$
		Mean	3.513 ± 0.185$^{(a)}$	2.928 ± 0.027$^{(b)}$	2.645 ± 0.026$^{(c)}$	1.617 ± 0.034$^{(d)}$
Fat (%)		Min	1.833 ± 0.058$^{(a)}$	1.467 ± 0.058$^{(b)}$	1.133 ± 0.057$^{(c)}$	/
		Max	3.500 ± 0.000$^{(a)}$	3.300 ± 0.000$^{(b)}$	3.067 ± 0.058$^{(c)}$	/
		Mean	2.733 ± 0.145$^{(a)}$	2.233 ± 0.051$^{(b)}$	2.075 ± 0,058$^{(b)}$	/
Minerals (mg/L)	Ca	Min	902.667 ± 2.517$^{(c)}$	1125.333 ± 1.102$^{(d)}$	1351.667 ± 1.528$^{(b)}$	2182.000 ± 6.557$^{(a)}$
		Max	1093.000 ± 36.592$^{(c)}$	1553.333 ± 2.082$^{(d)}$	1853.667 ± 3.215$^{(b)}$	2680.333 ± 4.509$^{(a)}$
		Mean	926.933 ± 116.002$^{(c)}$	1318.930 ± 2.910$^{(d)}$	1563.867 ± 1.665$^{(b)}$	2641.333 ± 3.202$^{(a)}$
	Na	Min	739.667 ± 2.887$^{(c)}$	1670.000 ± 5.196$^{(d)}$	2301.667 ± 2.082$^{(b)}$	8875.000 ± 5.000$^{(a)}$
		Max	943.000 ± 4.359$^{(c)}$	2115.670 ± 1.069$^{(d)}$	3343.333 ± 3.055$^{(b)}$	14012.333 ± 10.970$^{(a)}$
		Mean	826.800 ± 2.506$^{(c)}$	1823.000 ± 6.470$^{(d)}$	2683.000 ± 1.311$^{(b)}$	11501.200 ± 1.311$^{(a)}$
	K	Min	1723.000 ± 1.732$^{(b)}$	1596.677 ± 2.062$^{(d)}$	1623.667 ± 1.528$^{(c)}$	1807.667 ± 6.807$^{(a)}$
		Max	2185.333 ± 5.033$^{(c)}$	2310.000 ± 1.732$^{(d)}$	3051.333 ± 1.528$^{(a)}$	2957.333 ± 6.658$^{(b)}$
		Mean	1937.733 ± 2.344$^{(c)}$	1946.533 ± 4.005$^{(d)}$	2117.000 ± 1.249$^{(b)}$	2256.067 ± 4.081$^{(a)}$
	Cl	Min	4342.667 ± 3.215$^{(c)}$	4806.670 ± 0.577$^{(d)}$	6543.000 ± 2.646$^{(b)}$	10873.333 ± 3.055$^{(a)}$
		Max	8852.667 ± 2.517$^{(c)}$	8386.670 ± 0.577$^{(d)}$	19198.667 ± 576.486$^{(b)}$	45380.333 ± 0.577$^{(a)}$
		Mean	6263.000 ± 2.272$^{(c)}$	6602.330 ± 13.200$^{(d)}$	11755.580 ± 115.796$^{(b)}$	31546.667 ± 2.318$^{(a)}$

$^{a-d}$: Different letters in the line indicate a significant difference ($P < 0.05$) between the results of different camel samples. DM: Dromedary milk, DMU1: Dromedary Milk-Urine mixture 9–1 (v/v), DMU2: Dromedary Milk-Urine mixture 8–2 (v/v), DU: Dromedary urine.

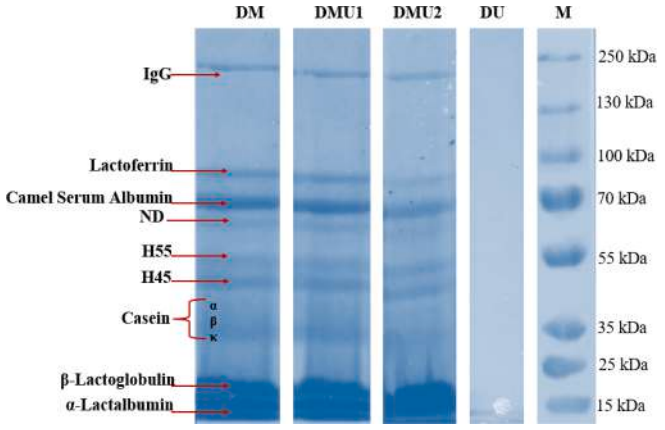

Fig. 3. Separation patterns of camel milk proteins on SDS-PAGE, DM: Dromedary milk, DMUA: Dromedary Milk-Urine mixture 9-1 (v/v), DMU2: Dromedary Milk-Urine mixture 8-2 (v/v), DU: Dromedary urine.

total absence of any protein (Lane 4, Fig. 3).

From the obtained SDS-PAGE nine major bands were observed, in which eight were identified in the three lanes of DM, DMU1, and DMU2 (Fig. 3) resolved from the migration of milk protein, the IgG with a weight of 210 kDa, Lactoferrin, Camel serum albumin and Heavy chains of immunoglobulins G (H55 and H45) with 87, 68, 55 and 45 as a molecular mass, respectively (Shuiep, 2015; Yasmin et al., 2020). The three-milk casein α, β and κ appeared in one large band starting from ≈37 kDa to ≈25 kDa supporting the results reported by Shuiep in 2015 (Shuiep, 2015), the two bands with a molecular weight of 18 kDa and 14 kDa represent the β-Lactoglobulin, and α-Lactalbumin, respectively (Yasmin, et al., 2020). However, the obtained results can't confirm the possible interactions or binding between DU elements and the DM proteins that can be resolved from mixing the two liquids, where the SDS-Page cannot give a clear vision on the structural changes or the interactions of the amino acids between milk and urines contents (Wittig & Schägger, 2005).

3.5. Protein content

The protein content of DM samples by the FTIR was 3.512 ± 1.85 %, in perfect agreement with the global average of the protein content in dromedary camel milk is approximate 3.03 % (G. Konuspayeva, Faye, & Loiseau, 2009), as well both the minimum and maximum values found in the milk samples (Table 2) are included in the general averages of the protein concentration in dromedary camel milk that varied between 2.15 % and 4.9 % (Hammam and Agriculture, 2019; Ho, Zou, & Bansal, 2021). Additionally, the obtained results are in a similarity to those reported by other studies carried out on the Algerian camel milk applying either spectrophotometric methods of biuret or Bradford or by the calculation of the total nitrogen according to the Kjeldahl method, where the protein content was found to go from 3 % to 3.5 % (Hadef, Aggad, Hamad, & Saied, 2018; Zahra et al., 2021). Moreover, the mean value of the protein content found in DM registered in Table 2 runs in the same flow as the results recorded by Mohamed et al (2021) with the use of near and mid-infra-red spectroscopy, yet with slight waving that is mainly related to the lactation stage, feeding and diet of the animals (food intake) which is connected to the season and the availability of the plant species in the desertic areas in which it reaches its peak of poverty in the period where the sampling was carried out (Musaad Mustafa, Faye, & Al-Mutairi, 2013; Yang, Yang, Yi, Pang, & Xiong, 2013). So far very few works are carried out on the evaluation of the protein concentration of DM using the FTIR that was proved to be a very useful and efficient method in this study due to its high sensitivity to detects protein's secondary structure (amide I and amide II bounds) guaranteeing by that a high determination of proteins concentration in dairy products (Grewal, Huppertz, & Vasiljevic, 2018).

The noted concentration of molecules with Amide I band in both mixtures DMU1 and DMU2 was not in total coordination with protein concentration in the milk sample after eliminating 10 % and 20 % of the milk proteins (Table 2), therefore the registrated amount does not include only milk proteins but also some of it comes from urine, in addition to the possibility of forming new amide bonds which can lead to influence milk proteins proprieties and capacities, especially that most of the value of the DM is linked directly proteins (Al-Juboori et al.,

2013).

DU presents a concentration varying from 1.24 ± 0,22 % to 2.27 ± 0,58 % with a mean of 1.62 ± 0,34 % of molecules containing the Amide I bound, the obtained concentrations by the FTIR scanning do not reflect the amount of proteins that were absent in the urine samples as the SDS-PAGE demonstrated (Lane 4, Fig. 3) but it corresponds to the molecules containing C═O group with starching capacity in 1600 cm-1 1700 cm-1 area producing by that a peak in this range such as peptides with a molecular weight<10 KDa, in addition to the nitrogenous molecule such as urea, uric acid, and creatinine that contains a C═O group (Sarigul, Kurultak, Uslu Gökceoğlu, & Korkmaz, 2021) presenting in low concentration in the DU due to the recycling proprieties of the kidneys of this animal that reuses the nitrogen of those molecules for the production of proteins and this is the reason that camel urines is considerate less or non-toxic to the consumers (Ahamad, Alhaider, Raish, & Shakeel, 2017).

3.6. Sugar content

As Table 1 presents, sugar concentration documented in the dromedary milk samples refers to the lactose content, the recorded percentage seems similar to the mean lactose content in dromedary milk in general and included in the range of the lactose content in the DM that varied between 2.4 % and 5.8 % as reported in precedent researches (Hammam and Agriculture, 2019; Ho, Zou, & Bansal, 2021). The recorded data showed a little ranging from the one reported by Mohamed et al (2021) using near and mid-infra-red spectroscopy, A variation mostly influenced by the external factors such as the availability of food and water that affect the production amount of lactose by the dromedary (Mohamed, Nagy, Agbaba, & Kamal-Eldin, 2021; Musaad Mustafa, Faye, & Al-Mutairi, 2013; Yang, Yang, Yi, Pang, & Xiong, 2013). In addition to its nutritive importance, the lactose content influences the teste and sensory acceptability of camel milk, from the obtained values of the studied DM samples can be classified as acceptable in terms of taste (Khaskheli, Arain, Chaudhry, Soomro, & Qureshi, 2004).

The level of sugar found in DU in this study is 2.04 ± 4,55 % a non-comparable value due to the lack of work on sugar content in camel's urine, but a very high amount compared to the urine of humans (15 mg/dl). From a veterinary point of view, the ten chosen animals were not diabetic cases and were perfectly healthy despite the level of sugar found in these animals' urine. Moreover, in a previous study realized by Ahamad et al. (2017) it was detected the presence of some sugars elements in DU that were galactose, D-galactose, melibiose, and 2-Deoxy-galactose produced from glycolysis pathways (Ahamad, Alhaider, Raish, & Shakeel, 2017); these results support the theory of the unique functionality of the dromedary kidneys and the different physiology of this animal comparing to other animals in general and mammals in specific (Tharwat, 2020).

The illustrated amount of the sugar element demonstrated by the two mixtures DMU1 and DMU2 was proportional to the removed percentage of lactose from the DM samples and the added content of the sugary elements of the DU. Through the obtained results of the sugary content in the DM, DMU1, and DMU2 from the FTIR scanning and calculation, this method can be categorized as an effective, specific, and sensitive technic for the determination of the sugar concentration in milk in specific and dairy food in general.

3.7. Fat content

The fat content of the different milk samples ranged from 2.47 ± 0.25 % to 3.50 ± 0.00 % (Table 2) with a mean value included in the global range of the fat content of the DM extending from 1.2 % to 6.4 % (Zibaee, Hosseini, Yousefi, Taghipour, Kiani, & Noras, 2015). However, the recorded concentration is slightly lower than those illustrated by precedent research, where the general amount of fats in camel milk was found to be 4 % (Ho, Zou, & Bansal, 2021). This difference is mainly due to the sampling period characterized by a very important lack of nutrition where it was demonstrated in several pieces of research that the seasons, nutrition, and water intake are factors that affect and change the milk compositions including the fat level of mammal milk in general (Yang, Yang, Yi, Pang, & Xiong, 2013). The two mixture samples demonstrated a significant drop ($P > 0.05$) in fat content relating to the eliminated quantity of milk and replaced by the DU (Table 2).

3.8. Mineral's content

As illustrated in Table 2. Four minerals were evaluated in the tested samples calcium, sodium, Potassium, and chloride. The detected amount of Ca in DM samples was found with a mean that corresponds to the data revealed in multiple research that extend from 880 to 1500 mg/L, a clear variation of the Ca content was noted in DM either in the tested samples or in the one documented previously (Gorban & Izzeldin, 1997) where it can't be confined in the very limited interval because it depends on many factors, the physiological state of the animal, water intake, season, and the feeding system it. Factors that can also cause a variation in Na and K content in DM that found in mean values similar to the amount documented in numerous studies where the Na concentration proximity varies from 600 to 950 mg/L and the K goes from 1270 mg/L to 1890 mg/L (Table 2) (Mostafidi, Moslehishad, Piravivanak, & Pouretedal, 2016; Sumaira et al., 2020). DM is classified as a rich source of electrolytes by offering the necessary amount needed to the human body (Konuspayeva, Narmuratova, Meldebekova, Faye, & Loiseau, 2008).

The chloride content in camel milk, in general, is not well investigated, according to the few literature data it was documented with an average concentration of 2500 mg/L (Alaoui Ismaili, Saidi, Zahar, Hamama, & Ezzaier, 2019; Khaskheli, Arain, Chaudhry, Soomro, & Qureshi, 2004), low concentration comparing to the recorded in this work that reached a value of 6263 mg/L associated with drought condition, feeding, lactation stage, and more importantly to the water quality consumed by the animal (Kashaninejad & Razavi, 2021).

A signific increase was documented for the four analyzed minerals in the DMU1 and DMU2 compared to the DM samples (Table 2), augmentation linked straightly to the added quantity of DU that illustrated a high concentration of the four analyzed minerals as Table 2 demonstrates. The increase in the mineral content for DMU1 and DMU2 can lead to a reverse result by influencing the general priorities of the milk generally and proteins specifically (Ocak and Rajendram, 2013).

The obtained values for the DU cannot be perfectly judged and evaluated due to the non-availability of previous data, yet the documented content of these minerals in the DU samples is directly related to two factors, dromedary kidneys functionality that produce ~~a~~ very concentrated urine, and the consumed water by the animal where it was highlighted that the undergrounded desertic water is saturated with minerals such as Ca, Na, K and chloride that can reach a value of 5000 mg/L in the southern regions of Algeria (Bouchekima, Bechki, Bouguettaia, Boughali, & Meftah, 2008).

3.9. Protein oxidation

As presented in Fig. 4, A significant difference was recorded between the same samples type ($P > 0.05$). The lowest concentration of the sulfhydryl group (-SH) was demonstrated in DM, and the highest -SH groups were in DM2 reflecting the highest protein oxidation. The level of the –SH was significantly increased with the addition of the DU to the milk ($P < 0.05$), the more urine is added the more protein are oxidable by breaking the SS bounds of the cysteine amino acid of the DM proteins, this can be due to the high menial content of the DU (Table 2), alkaline pH (Table 1) or by other compounds of the DU that makes DM protein easily oxidated (Shacter, 2000).

The structure, solubility, mobility, and functionality of milk proteins are maintained by the SS bridges. Breaking these bound results, the loss

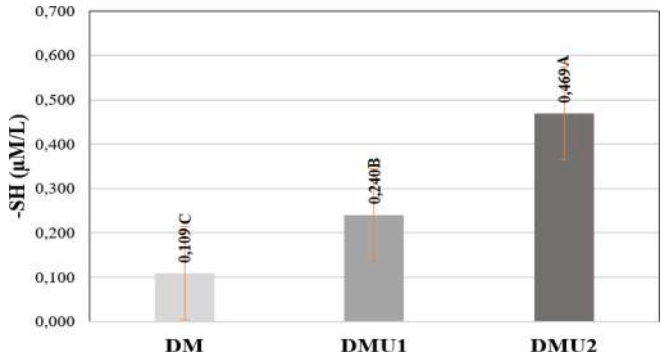

Fig. 4. Sulfhydryl groups resulted from protein oxidation in DM, DMU1, and DMU2. $^{A-C:}$ Different letters indicate significant difference ($P < 0.05$) between the results of the different samples.

of the general conformation of the milk proteins, exposing their hydrophobic domain, and some reactive groups enhancing by that the rheological, and physiological proprieties of the milk (Hawkins and Davies, 2019). Moreover, it has been reported that oxidation of milk protein linked to the -SH groups causes a remarkable decrease in the digestibility of milk caseins that compose 80 % of the total milk protein (Guha et al., 2021). Most of the bioactive peptides resulting from DM digestion are generated from the hydrolysis of the caseins, where the more casein are digested the more peptides with different therapeutical potentials are produced such as antioxidant ability either in scavenging free radicals or in reducing Fe iron, ACE inhibition capacity, antidiabetic, and antiobesity properties all from the proteolysis of DM casein by pepsin, chymosin, and trypsin (Guha et al., 2021) that can be reduced by the loss of casein structure caused by the oxidation of its disulfides groups due to the addition of DU.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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RESEARCH ARTICLE

The effect of the Feeding system on the Antioxidant activity of Camel urine

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ABSTRACT:
Camel urine has been widely used in the biomedical field as a traditional healing liquid for several health disorders, this study aims to evaluate the antioxidant activity of camel urine and its association with the breeding and the feeding system. Urine samples were collected from domestic (from private farms) and desertic camels, where spectrophotometric method was chosen to evaluate the phenolic, flavonoid content and the antioxidant activity. As results, it was found from the applied testes that the antioxidant activity of the camel urine is very important, where both types of urine illustrated a very low EC50. However, it has been found that the significant anti-radical activity and a reducing power of urine of domesticated camels fed in private farms was higher than the urine of desertic camels. concluding that the consumption and usage of camel urine can contribute to the prevention of diseases associated with oxidative stress.

KEYWORDS: Camel urine, Domesticated camels, Desertic camels, Phenolics, Antioxidant.

INTRODUCTION:
The use of natural products to treat pathologies and several health disorders is nowadays a very popular means. Besides medicinal plants, human and animal urine are used in traditional medicine under the name of urine-therapy with the using of some the human urines[1] or animal urines to heals some health disorders such as cow and camel[2,3]. Camel" Camelus dromedaries" urine (CU) has been widely used in the biomedical field in several Arabian countries such as Algeria especially in the Saharan regions as healing biological liquids for wounds and burns, against eczema and allergies, anti-dermatophytes and antitumor, without harmful side effects[4,5],

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where of the effected researches and biochemical analysis and studies carried out on CU that focused on nitrogen metabolites has found that those metabolites are in very low concertation comparing to other type of urine making CU less or no toxic[6].

So far, a few activities of CU have been revealed such as its capacity to inhibit the proliferation of some bacterial, fungal and viral strain due to its chemical composition[4,7]. Also, CU has demonstrated anti-cancer properties[8] by blocking the expression of some genes that activate cancer at the transcriptional and post-transcriptional stages[9]. Moreover, CU can play the role of an antiplatelet agent[10] and in treating some diabetic complications[11]; it was also found that camel urine has an efficiency against multi-drugs resistant Pseudomonas aeruginosa, isolated from effected burns, wounds and ears[12]. However, though the potential capacities that CU may illustrate it didn't take an important place in the

traditional pharmacology researches unlike cow urine that took a wide place in literature researches[13,14].

The therapeutic forces exhibited by this urine may be directly related to the richness of Saharan plants species grazed by the dromedary camel with active ingredients such as second metabolites, where these components are believed to be the source of the biological activities of this liquid[15], On the other hand, the uniqueness of this animal and its physiological structure can be the reason for the utility and capacities of its urine.

Thus, this study aims to evaluate the antioxidant activity of camel urine carried out under two breeding and feeding system domesticated and desertic.

MATERIALS AND METHODS:

Sampling:

The urine used in this work comes from non-lactating, disease-free, aged between 2-8years female camel (camel dromedaries) belong to the Sahrawi breed, from Ouargla, Algeria. Urine samples were collected from camels under two different breeding systems (five of each):

- Domesticated camels (DC), raised on a private farm where they had free access to water and food such as cereal grain, some plants cut and dried bread.
- Desertic (Saharan) camels (SC), desert living that fed only on the Saharan plants with the lack of water.
- CU was collected in the morning (4-5am) with experienced help in dark, sterile vials and immediately transported in coolers at 4°C to the laboratory to be lyophilized for the study assays.

Phenolic compounds determination:

The determination of the total phenolic compounds was carried out using the Folin-Ciocalteu reagent, applying the coulometric assay reported[16].

A quantity of 100µl of each solution is introduced using a micropipette into test tubes, followed by the addition of 500µl of the Folin-Ciocalteu reagent (diluted 10 times). After incubation for 2min, 2ml of 20% sodium carbonates (Na_2CO_3) is added, then kept in the dark for 30min at room temperature. The absorbance of each solution was determined at 760nm against a blank prepared in the same manner except that it did not contain gallic acid. The standard calibration curve was obtained from gallic acid solutions of different concentrations (0.01-0.3mg/ml), where the optical density readings at 760nm of the solutions thus prepared allowed the calibration curve for gallic acid to be drawn [17].

Flavonoids Determination:

The determination of flavonoids was carried out using the aluminum trichloride ($AlCl_3$) method described by[18]. To a volume of 1ml of each urine 1ml of the $AlCl_3$ solution (2% in methanol) was added. After 10 minutes of incubation, the absorbance measuring was affected at 430nm. Using the rutin as a standard at different concentrations (0-10mg/L), for the calibration curve obtaining, following the same steps of the assay. The results were expressed in mg Rutin equivalent (RE) per liter of urine volume (mg RE/ LU)[19].

Fourier-transform infrared spectroscopy (FTIR) characterization:

The equipment used for this study was the Bruker Tensor 27 spectrometer equipped with a DLA TGS detector (Bremen - Germany), and the data acquisition was accomplished using OPUS program version 7.2 for Windows from Bruker GMBH. The studied products were directly injected and compressed on the ATR crystal surface using distilled water as background, where the FTIR scanning was performed at a band interval of 4000-600 s-1, accumulating 16 scans per spectra.

Antioxidant capacity evaluation:

Four testes were chosen for the oxidation inhibition activity as following:

a) DPPH scavenging assay:

A volume of 0.05ml of different concentrations of each urine was added to 1.950ml of methanolic solution of DPPH (0.024 g/l). the 2ml solution was then incubated in the dark for 30min and at room temperature, the absorbance reading was taken at 515nm. The inhibition percentages were calculated by the following formula:

$$I\% = ((Ab - AS)/ Ab) *100$$

Where: AS is the samples absorbance, Ab is the blank absorbance[20].

b) ABTS scavenging assay:

A 16h earlier prepared stock solution of ABTS was diluted with phosphate buffer (0.2 M, pH 7.4) to obtain an absorbance of 0.70 ±0.2 at 743 nm. From this solution a volume of 1.95 ml was mixed with 0.05 ml of urine at different concentration and kept for 6-7 min in obscurity at room temperature than the absorbance was recorded at the same wavelength mentioned before using phosphate buffer as blank[21]. Inhibition ration percentage was obtained using the following formula:

$$I\% = ((Ab - AS)/ Ab) *100$$

Where: AS is the sample absorbance, Ab is the blank absorbance.

c) FRAP reduction test:

Applying the spectrophotometric assay reported by[22] this test was accomplished. The absorbance of the final obtained solution is determined at 700nm, where the more the absorbance is high the more is the reducing power of the tested samples. This solution is obtained by mixing a volume of 1ml of urine at different concentrations with 2.5ml of PBS (0.2M, pH 6.6) and the same volume of potassium ferricyanide $K_3Fe(CN)_6$ solution (1% W/V). this mixer was put in a water bath of 50°C for 20 min. Another volume of 2.5ml of trichloroacetic acid (10% W/V) was added to end the reaction and the tubes were centrifuged at 3000rpm for 10min, a 2.5ml of the supernatant was combined with 2.5ml of distilled water and 0.5ml of aqueous FeCl3 solution 0.1% (W/V). similarly, prepared blank, replacing the urine with distilled water which makes it possible to calibrate the device (UV-VIS spectrophotometer)[23].

d) PPM (Phosphomolybdate) assay:

The method involves placing in a hemolysis tube 200µl of urine at difrent concentration mixed with 1800µl of a reagent composed of H_2SO_4 (0.6 M), NaH_2PO_4 (28 mM) and ammonium molybdate (4mM). The tube is then tightly closed and then incubated at 95°C. for 90 minutes in a water bath. After cooling the mixtures to room temperature, the absorbance is measured at 765 nm. the reducing power increasing is aligned with the absorbance increasing[21]. Reducing power was estimated using following formula:

Reducing power (%) = [(Ab - As)/Ab] * 100

Where: AS is the sample absorbance, Ab is the blank solution absorbance.

Statistical analysis:

Using the SPSS package program (IMB SPSS 25) Statistical analysis was carried out. Preforming a One-way analysis of variance (ANOVA) and the statisticaldifferences between the means were evaluated at the significance level of 0.05 using the Tukey test. The experimental analyses were performed in triplicate.

RESULT:

Phenolic and flavonoids content:

As presented in Table 1 phenolic compounds are reported in mg gallic acid equivalent per liter of urine. The urine of the SC illustrated a content of phenolic compounds significantly higher ($p<0.05$) comparing to the urine of the DC with an average of 0.84±0.01mg GAE/LU that was 0.45±0.04mg GAE/ LU in the (Table 1).

The quantification of flavonoids was carried out by a standard solution (Rutin) at different concentrations. The results obtained are shown in Table 1, where the flavonoid content in the urine of camels carried out in the desert was 0.014±0.003mg RE/LU, while the second type of urine contained a concentration of 0,005±0.001

Table 1. Total phenols (mg GAE/UL) and total flavonoids (mg RE/U L) in both domesticated and desertic camel urine

Samples	Total phenolics (mg GAE/ UL)	Total flavonoids (mg RE/ UL)
SC1	0.87 ± 0.03^{a}	0.011 ± 0.001^{B}
SC2	0.89 ± 0.03^{a}	0.013 ± 0.001^{B}
SC3	0.77 ± 0.04^{b}	0.016 ± 0.001^{A}
SC4	0.75 ± 0.03^{b}	0.017 ± 0.002^{A}
SC5	0.90 ± 0.04^{a}	0.016 ± 0.001^{A}
DC1	0.45 ± 0.01^{cde}	0.004 ± 0.000^{C}
DC2	0.48 ± 0.02^{cd}	0.006 ± 0.000^{C}
DC3	0.51 ± 0.02^{c}	0.005 ± 0.001^{C}
DC4	0.42 ± 0.02^{de}	0.004 ± 0.000^{C}
DC5	0.41 ± 0.02^{e}	0.006 ± 0.001^{C}

The values are the mean of three determinations ± standard error.
a-e: Different letters in the column indicate a significant difference ($P<0.05$) between the results of different camel samples; A-C: Different letters in the line indicate a significant difference ($P<0.05$) between the results of same camel sample.

Fourier-transform infrared spectroscopy (FTIR) characterization:

From Figure 1 and 2, both FTIR scans gave practically the same clear peaks pattern of the functional groups that exist in the studied samples, yet with an interval of 2 to 3 cm-1 between the peaks profile of the two types of urine which account insignificant.

From the obtained graphs profile the following functional groups where revealed (Figure 1,2), starting with a large band referring to the symmetric and asymmetric elongation of the hydroxyl group "OH" at ~3440 cm−1, followed by a peak at ~1630 cm−1 representing the NH corresponds to the nitrogen molecules and the carbonyl C=O groups that of urines. Two small successive bands at ~1399 cm−1 ~1460 cm−1 relating to the N-H deformation and the C=C stretching cm−1 causing by the to the hypochromic action of the amid and carbonyl groups and at ~1245 cm−1 ~1300 cm−1 bands at corresponding to the C-O group, with a high peak that was at ~1100 cm-1 relating to the C-O group and the C-N vibrations[22-24].

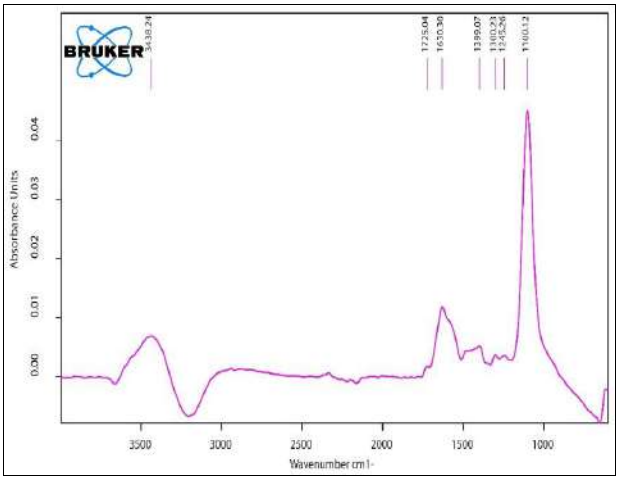

Figure 1: FTIR absorption spectrum of urine obtained from domesticated camels (DC).

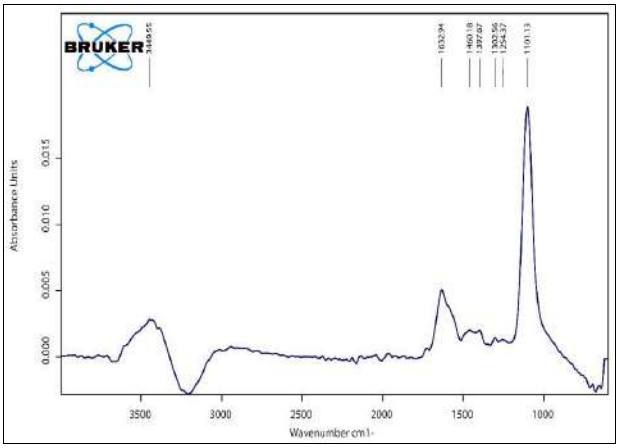

Figure 2. FTIR absorption spectrum of urine obtained from desertic camels (SC).

Antioxidant capacity evaluation:

The antioxidant activity of camel urine was investigated using four tests, ABTS and DPPH radical scavenging tests, with the phosphomolybdate and FRAP test that allowed us to assess the antioxidant status of camel urine. It appears from the results of the four testes that the antioxidant activity of the studied product is very important compared with the standard (ascorbic acid).

From Figure 3, it can be seen that the effective concentration to inhibit 50% of free radical's DPPH by the SCU, DCU, and AA was 19.133±0.402, 15.467±2.411 and 6.427±0.369µg/ml respectively. Where both urines showed a great oxidation inhibition capacity, yet the anti-free radical activity of the urine of camels carried out in the private farms was significantly higher ($p<0.05$) than the ability demonstrated by the urine of camels carried out in the desert. the same results were illustrated in the ABTS scavenging test where the half maximal effective concentration (EC 50) value of the DCU was almost twice lower than ($p<0.05$) the EC50 of the SCU with a value of 16,80±0,382 and 9,600 ±1,992 for the SCU and DCU, respectively (Figure 3).

According to Figure 3, the reducing powers of the samples in the PPM and the FRAP tests of both tested samples was interesting, yet the reducing power of both urine in the FRAP test was more important with a very low concentration than the PPM test where the EC50 of the urine from camels conducted in the domesticated farms was 85,467±5,084 and 102,596±1,853 for the urine of the desertic camels ($p<0.05$).

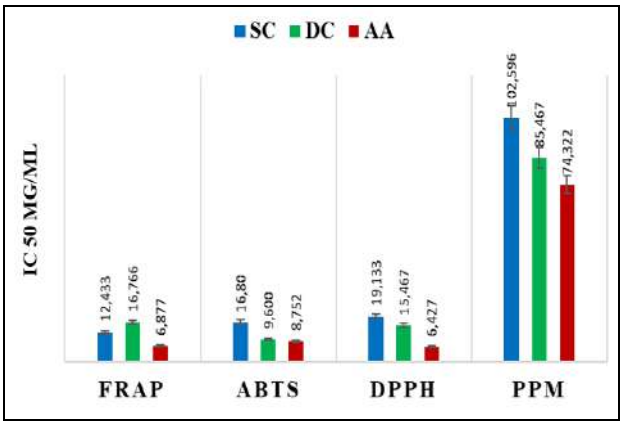

Figure 3. Half maximal effective concentration (EC50) values of DC and SC urine and the standard. ANOVA, with Tukey test with *p < 0.05 (n=5, ±SD).

DISCUSSION:

The variability of the contents of phenolics and flavonoids in the two types of urine analyzed is linked to the physiological state of the animal and to the phenolic composition of the food ingested, part of which is eliminated mainly in the urine[28,29]. Indeed, where the urine of the SC showed a higher concentration of those compounds due to the richness of plants or the nutrients ingested in these compounds[30,31]. Unlike the urine of the domesticated camels that showed a very low level of both phenolics and flavonoids due to the lack of their ingested food of those elements where their diet is mainly based on fodder that is exclusively cereal grain, some plants cut and dried bread, the data analysis reveals that the feeding system is related to the concentration of phenolics and flavonoids in both urine type.

The FTIR characterization showed that both of the tested urine demonstrated the same peak pattern which means the composition of the DC and SC urine is almost the same despite the feeding system difference, concluding that no matter what the feeding and breeding method is the camel kidneys will exhibit the same recycling and filtration processes.

The antioxidant capacity demonstrated by both type of urine can be related to the presence of the "OH" functional group as shown in the FTIR profiles of the SCU and DCU (Figure 3) which is widely known with

its high capacity as a reducing and scavenging group[32]. Moreover, the oxidation inhibition ability can be also related to the presence of phenolic compounds in the two studied urine that is classified as the most powerful agents to trap the free radicals gents[33], yet the very low concentration of this compounds is insufficient to explain this important activity, especially that the concentration of those elements was lower in the DCU yet this urine showed a higher antioxidant capacity comparing to the SCU.

Moreover, it appears from the obtained results by all applied tests, that the significant anti-radical activity and reducing power of urine of camels conducted in the private farms was higher than the urine of camels conducted in the desert (Figure 3), which was not proportional to the phenolic compounds content in both studied urines that was higher in the urine of the desertic camels, suggesting that this activity is note related to the phenolic compounds concentration. Moreover, from the obtained absorbance of the FTIR spectrum, it appears that the concentration of the functional groups in the urine of DC is higher than the urine of the SC due to the free access of food and water unlike the other camels, where the more this animal is fed the more the secretion of the molecules with antioxidant ability is high which denied the theory that refers the CU value to the richness of the desertic plants consumed by this animal with active elements, which means that the oxidation inhibition ability demonstrated by CU is mainly related to the physiological, biological and metabolic characteristics of the Camelus dromedaries which can influence the pathways of the biosynthesis of this biological fluid by producing molecules responsible of activity in this urine[34].

The consumption of camel urine with high antioxidant power can therefore be applied for the prevention of diseases associated with oxidative stress such as cancer, digestive disorders, and cardiovascular diseases[33,35].

CONCLUSION:

The results of the executed study demonstrate the existence of a small amount of phenolics and flavonoids where the concentration was proportional to the camel feeding system, it was also revealed from this work that the urine of the Camelus dromedaries exhibit a very imported antioxidant capacity no matter what the breeding method and the feeding system was desertic or domesticated, therefore the consumption and usage of camel urine with high antioxidant activity can contribute to the prevention of diseases and health disorders associated with oxidative stress.

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation**.**

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