Lipase inhibitory activity of Georgian wines

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A dissertation

submitted to the dissertation council of the Agrarian Sciences

of the Agricultural University of Georgia as a partial fulfilment of the requirements

for the degree of Doctor of Philosophy in Agrarian Sciences

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Agricultural University of Georgia

Tbilisi, 2022

სამეცნიერო მიმართულების კომისიის რეკომენდაცია

დისერტანტი: ჟუჟა ხაჭაპურიძე

დისერტაციის სათაური: „ქართული ღვინოების ანტილიპაზური აქტივობა“ / „Lipase Inhibitory Activity of Georgian Wines”

დისერტაციის დაცვის თარიღი: 28 ოქტომბერი, 2022

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I declare that this dissertation is an original report of my research, was composed solely by myself, and the work has not been submitted for any other degree or professional qualification. The experimental work contained herein is entirely my work. I confirm that the work submitted is my own, except where work which has formed part of jointly authored publications has been included. My contribution to this work and those of the other authors have been explicitly indicated below. Appropriate credit has been given within this thesis where reference has been made to the work of others. Part of this work was previously published:

In the Ukrainian Food Journal as "*In-vitro functional efficacy of extracts from Caucasian Rhododendron (Rhododendron caucasicum) and Rkatsiteli wines as pancreatic lipase inhibitors*", by Zhuzha Khatchapuridze, Givi Gugulashvili, Vitali Ghvachliani, Angelika Ploeger, Levan Gulua, and Tamar Turmanidze (DOI: 10.24263/2304-974X-2021-10-1-4). In the Annals of Agrarian Science as "*Comparative study of lipase inhibitory activity of some Georgian wines obtained through Kakhetian and European winemaking techniques*" by Z. Khatchapuridzea, A. Ploeger, L. Gulua, and T. Turmanidze.

Part of this work will also be published in Food Research as "*Kinetic Behaviour of Pancreatic Lipase Inhibition by Wine*" by Z. Khatchapuridzea, A. Ploeger and L. Gulua. The following manuscript has been accepted for publication and is on the waiting list for the technical review. The abovementioned studies were conceived by all the authors. I was involved in performing experiments, processing, and interpreting data, along with preparing, writing, and revising the manuscripts.

Zhuzha Khatchapuridze

September, 2022

ჟუჟა ხაჭაპურიძის

სადისერტაციო ნაშრომის

ქართული ღვინოების ანტილიპაზური აქტივობა

ანოტაცია

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პროფ. ლევან გულუა

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ასისტენტ პროფ. თამარ თურმანიძე

სიმსუქნე, რომელიც მიჩნეულია ოცდამეერთე საუკუნის დაავადებად და „ახალი სამყაროს სინდრომად“, საზოგადოებრივი ჯანმრთელობის საყოველთაო პრობლემას წარმოადგენს. სხვადასხვა კვლევამ აჩვენა, რომ სიმსუქნის გამომწვევი მიზეზი ხშირად ცხიმებით მდიდარი კვებითი რაციონია. საკვებისმიერი ცხიმების შეთვისება დამოკიდებულია პანკრეატული ლიპაზას (EC3.1.1.3) მოქმედებაზე. აქედან გამომდინარე, ცხიმების შეთვისების შემცირება პანკრეატული ლიპაზის აქტივობის დათრგუნვით ხელსაყრელი სტრატეგიაა სიმსუქნის სამკურნალოდ. ამასთანავე, ლიპაზას ინჰიბირება არ იწვევს ორგანიზმში რაიმე მნიშვნელოვან სახიფათო გვერდით ეფექტებს.

დღეისათვის ამ მექანიზმით მოქმედი ერთადერთი კლინიკურად დამტკიცებული ფარმაკოლოგიური პრეპარატი არსებობს ბაზარზე, სავაჭრო ნიშნით ქსენიკალი და ალი (აქტიური ნივთიერება ორლისტატი), რომელსაც ყოველდღიურად მიღებული ცხიმის დაახლოებით 30%-ის მონელების შეფერხება შეუძლია. თუმცა, ამ მედიკამენტის მიღება დაკავშირებულია სხვადასხვა არასასურველ ეფექტთან.

შესაბამისად, აქტიურად მიმდინარეობს კვლევები ლიპაზას ახალი ბუნებრივი ინჰიბიტორის აღმოსაჩენად, რომელსაც მინიმალური გვერდითი ეფექტები და მაღალი ინჰიბიტორული აქტივობა ექნება.

უამრავმა კვლევამ აჩვენა, რომ პოლიფენოლების კლასი წარმოადგენს პანკრეატული ლიპაზის აქტივობის ინჰიბირების ერთ-ერთ ყველაზე მნიშვნელოვან წყაროს. ღვინო, თავის მხრივ, მდიდარია პოლიფენოლებით. ტრადიციული ქართული (კახური) მეთოდი, ღვინოს კიდევ უფრო ამდიდრებს ამ ნაერთებით. მიუხედავად იმისა, რომ ქართული და უცხოური ღვინოების შესახებ უამრავი კვლევაა ჩატარებული, ჩვენს ხელთ არსებული ინფორმაციით, მათი ანტი-ლიპაზური აქტივობის შესახებ მწირი ინფორმაციაა ხელმისაწვდომი.

კვლევის მიზანი: ზემოთხსენებულიდან გამომდინარე, აღნიშნული კვლევა მიზნად ისახავდა კომერციულად ხელმისაწვდომი ქართული ღვინოების ანტილიპაზური აქტივობისა და ბიოაქტიური ნაერთების შემცველობის შეფასებასა და შედარებას. წინამდებარე კვლევის კიდევ ერთ მიზანს წარმოადგენდა ღვინის მიერ პანკრეატული ლიპაზის აქტივობის ინჰიბირების მექანიზმისა და კინეტიკის დადგენა. ასევე, შესწავლილი იქნა ღვინის ცალკეული ფენოლური ფრაქციების მიერ ლიპაზას ინჰიბირების მექანიზმი და კინეტიკა.

კვლევა ითვალისწინებდა შემდეგ **ამოცანებს:**

- ღვინის ნიმუშების ძირითადი ხარისხობრივი და ბიოქიმიური მაჩვენებლების ანტილიპაზურ აქტივობასთან კორელაციის შესწავლა;
- ღვინის, როგორც ინჰიბიტორის ფრაქციონირება ეთილაცეტატისა და წყლის ფრაქციებად და მათი ინჰიბიტორული აქტივობის შესწავლა;
- ფერმენტის ინჰიბირების კინეტიკისა და მექანიზმის შესწავლა შერჩეული ღვინის და მისგან გამოყოფილი ფენოლური ფრაქციების მიერ;

- ფენოლური ფრაქციების, როგორც ლიპაზას ინჰიბიტორების, ურთიერთგავლენის შესწავლა;
- *In vivo* პირობებში ღვინის ანტილიპაზური აქტივობის შეფასება;

კვლევის სამეცნიერო სიახლე: ჩვენს ხელთ არსებული ინფორმაციით, ამ შინაარსის კვლევები აქამდე არ ჩატარებულა. ასე რომ, ღვინის მიერ პანკრეატული ლიპაზას ინჰიბირების მექანიზმისა და კინეტიკის გამოკვლევა წარმოადგენს ჩვენი ნაშრომის სიახლეს.

დისერტაციის სტრუქტურა

დისერტაცია მიჰყვება ძირითად სტრუქტურას და მოიცავს ხუთ თავს - შესავალს, სამეცნიერო ლიტერატურის მიმოხილვას, კვლევის მეთოდოლოგიას, შედეგებსა და მათ განხილვას და დასკვნებს.

ლიტერატურის მიმოხილვის ნაწილში განხილულია სიმსუქნის პრობლემა, სიმსუქნის მკურნალობისა და მართვისთვის გამოყენებული სტრატეგიები. ამას გარდა, ნაჩვენებია სიმსუქნეს კავშირი მდგრადი განვითარების მიზნებთან (SDGs) და განხილულია პანკრეატული ლიპაზას ფერმენტის როლი სიმსუქნესთან დაკავშირებით. ამავე თავში მიმოხილულია პანკრეატული ლიპაზას ბუნებრივი ინჰიბიტორები და ნაჩვენებია ქართული ღვინის პერსპექტივა ამ მიდგომის მიმართ.

კვლევის მეთოდოლოგიის ნაწილი იძლევა მკაფიო ახსნას შერჩეული მეთოდებისა და ტექნიკის ვარგისიანობისა და სანდოობის დასაბუთებით. დეტალურად არის აღწერილი კვლევის ობიექტები, გამოყენებული ინსტრუმენტები, პროცედურები, ექსპერიმენტის ჩატარების პირობები და მონაცემები, მათი ანალიზის მეთოდები. ღვინის მჟავათა საერთო რაოდენობის გამოსახატად განსაზღვრულია ტიტრული

მჟავიანობა ღვინის მჟავაზე გადაანგარიშებით. ჯამური ფენოლების შემცველობა განსაზღვრულია ფოლინ-ჩიკოლტეოს რეაქტივით, გალის მჟავაზე გადაანგარიშებით. ღვინის ნიმუშების ანტიოქსიდანტური აქტივობა განსაზღვრულია სპექტროფოტომეტრული FRAP (Ferric Reducing Antioxidant Power) მეთოდით. ფერმენტ ლიპაზას აქტივობა და ნიმუშების მიერ მისი ინჰიბირება გამოკვლეულ იქნა ტიტრაციის მეთოდით, სიგმა-ალდრიხის მიერ შემუშავებული მეთოდიკის მიხედვით. თხევად-თხევადი ექსტრაქციის მეთოდის საშუალებით მიღებულ იქნა ღვინის ფენოლური ფრაქციები. ფერმენტის ინჰიბირების კინეტიკა და მექანიზმი შესწავლილ იქნა ლაინუივერ-ბერკის გრაფიკული მეთოდის მიხედვით. კინეტიკური მონაცემების ანალიზი გამოისახა გრაფიკულად მიხაელის-მენთენის განტოლების შებრუნებულ კოორდინატთა სისტემაში, რის საფუძველზეც დადგინდა ინჰიბირების ტიპი. მონაცემები დამუშავდა Microsoft Excel-ით (Microsoft 365 MSO, Version 2112, statistical functions, Microsoft Corp., Redmond, WA, USA). წარმოდგენილი მონაცემები არის მინიმუმ სამი გამეორების საშუალო ± სტანდარტული გადახრა (SD).

მიღებული ექსპერიმენტული მონაცემები და კვლევის შედეგები განხილულია შედეგებისა და დისკუსიის თავში. ასევე განხილულია ზოგიერთი SDG-ის წარმატებით განხორციელების მნიშვნელობა. საბოლოოდ, მიღებული შედეგების ინტერპრეტაციის საფუძველზე მომზადდა დასკვნა.

კვლევის მნიშვნელოვანი შედეგები

წინამდებარე ნაშრომში გამოვიკვლიეთ 14 სხვადასხვა ქართული კომერციული ღვინის ინჰიბიტორული პოტენციალი პანკრეატული ლიპაზის მიმართ. მათ შორის, შესწავლილ იქნა მუკუზანის ღვინისა და მისი ფენოლური ექსტრაქტების მიერ ლიპაზას დათრგუნვის მექანიზმი და განსაზღვრულ იქნა შესაბამისი კინეტიკური პარამეტრები. შესწავლილი ღვინის ნიმუშები დამზადებული იყო ქართული ყურძნის ჯიშებისგან (საფერავი და რქაწითელი), კახური ან ევროპული მეთოდის

მიხედვით. კვლევის შედეგები იძლევა მტკიცებულებას, რომ წითელი ღვინო არის პანკრეატული ლიპაზის ძლიერი ინჰიბიტორი, შერეული ტიპის (კონკურენტულ-არაკონკურენტული) ინჰიბიტორული მექანიზმით.

$$
\begin{array}{ccc}
E + S & \leftrightarrow & ES \rightarrow E + P \\
+ & & + \\
I & & I \\
K_{i1} \updownarrow & & K_{i2} \updownarrow \\
EI + S & \leftrightarrow & EIS
\end{array}
$$

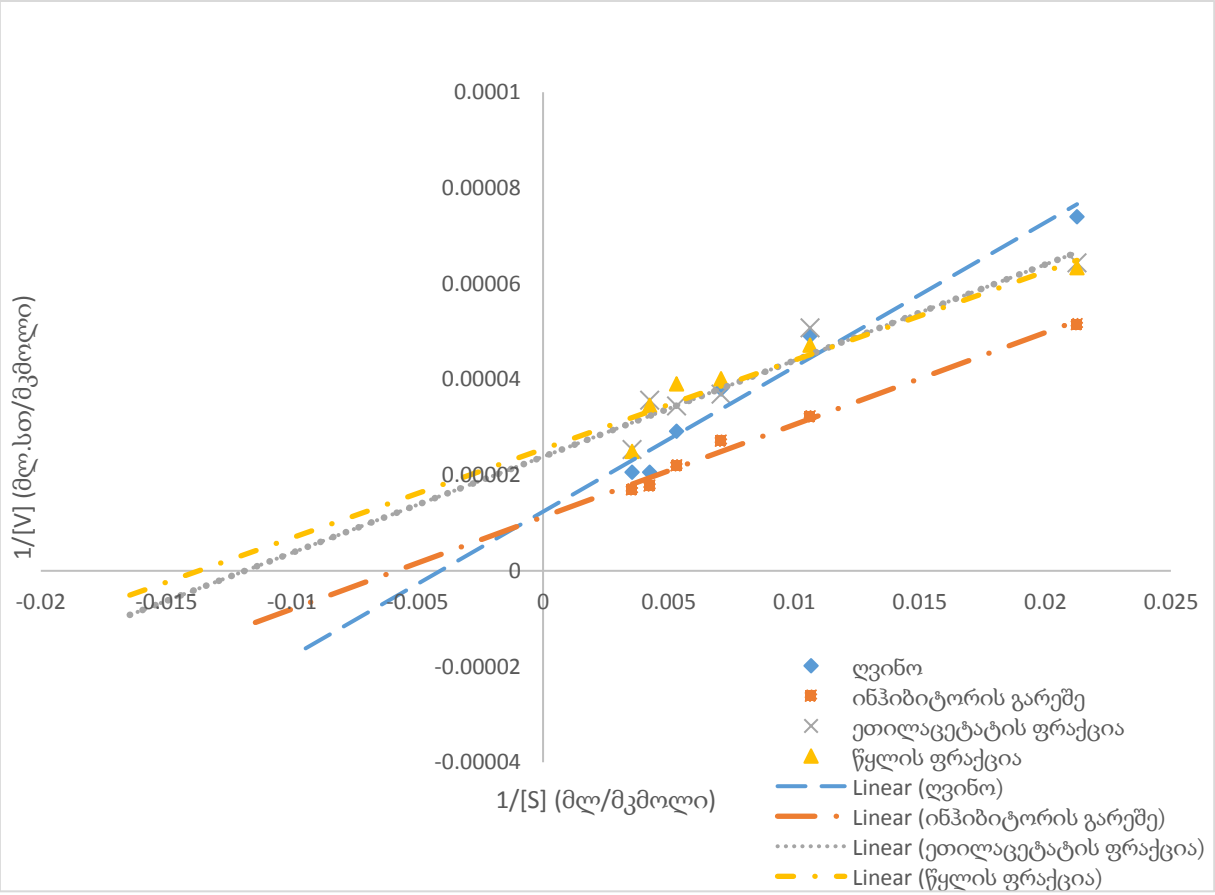
სქემა 1. შერეული ტიპის ინჰიბირების სქემა. [E]: ფერმენტი; [S]: სუბსტრატი; [I]: ინჰიბიტორი; [P]: პროდუქტი. ღვინო როგორც ინჰიბიტორი (I) უკავშირდება თავისუფალ ფერმენტს და წარმოქნის ფერმენტ-ინჰიბიტორის კომპლექსს (EI), ფერმენტ-სუბსტრატის (ES) კომპლექსთან დაკაშირებისას წარმოქნის ფერმენტ-ინჰიბიტორ-სუბსტრატის (EIS) კომპლექსს.

ინჰიბიტორმა შეძლო როგორც ფერმენტის, ასევე, ფერმენტ სუბსტრატის კომპლექსის შებოჭვა. შედეგებმა აჩვენა, რომ ინჰიბირების მუდმივას მნიშვნელობა (K_{i1}) ღვინის ფერმენტთან დასაკავშირებლად უფრო მცირე იყო, ვიდრე ინჰიბირების მუდმივა (K_{i2}) ღვინის ფერმენტ-სუბსტრატის კომპლექსთან დასაკავშირებლად, 40.556 ± 1.932 და 179.361 ± 8.678 $\mu mol \cdot mL^{-1}$, შესაბამისად. მონაცემები მიუთითებს, რომ ღვინის სწრაფვა თავისუფალი ფერმენტის მიმართ ბევრად უფრო მაღალი იყო, ვიდრე ფერმენტ-სუბსტრატის კომპლექსის მიმართ, რაც თავის მხრივ, აძლიერებს ინჰიბიტორულ ეფექტს.

ღვინის ფენოლური ნაერთების ეთილაცეტატისა და წყლის ფრაქციებმა, უკონკურენტო მექანიზმით დათრგუნეს პანკრეატული ლიპაზას აქტივობა.

ამასთანავე, აღნიშნულმა ფრაქციებმა გამოავლინეს სინერგიული ეფექტი ლიპაზას აქტივობის დათრგუნვის პროცესში.

სურ. 1-ზე ნაჩვენებია პანკრეატული ლიპაზას აქტივობის გრაფიკები ლაინუივერ-ბერკის კოორდინატებში, როცა სუბსტრატად ზეითუნის ზეთია გამოყენებული, ინჰიბიტორის თანაობისას და მის გარეშე.



სურ. 1. პანკრეატული ლიპაზას აქტივობის გრაფიკები ლაინუივერ-ბერკის კოორდინატებში, ინჰიბიტორის თანაობისას და მის გარეშე, სუბსტრატად გამოყენებულია ზეითუნის ზეთი.

კვლევებმა აგრეთვე აჩვენა, რომ კახური მეთოდით დამზადებული თეთრი ღვინოების ანტილიპაზური აქტივობა საგრძნობლად აღემატება ევროპული მეთოდით დამზადებული თეთრი ღვინოების ანტილიპაზურ აქტივობას.

ამასთანავე, კახური მეთოდით წარმოებულ თეთრ ღვინოებში ანტიოქსიდანტური აქტივობა და ჯამური პოლიფენოლების შემცველობა მნიშვნელოვნად მაღალია, ვიდრე ევროპული მეთოდით მომზადებულ თეთრ ღვინოებში. კახური სტილის ღვინოები დიდი ხნის განმავლობაში კონტაქტშია ყურძნის კანთან, კლერტთან და წიპწასთან ნაწილებთან, რაც განაპირობებს ბიოაქტიური კომპონენტების მიგრაციას ღვინოში.

ვერ აღმოვაჩინეთ მნიშვნელოვანი კორელაცია (R^2=0.4407) ღვინის ნიმუშებში პოლიფენოლების შემცველობასა და ანტილიპაზურ აქტივობას შორის, ვერც ღვინის მიერ ლიპაზას ინჰიბიტორულ აქტივობასა და ღვინის დაყენების მეთოდს შორის.

საერთო ჯამში, შეიძლება დავასკვნათ, რომ ჩვენ მიერ გამოკვლეული ღვინის ნიმუშების უმეტესობა პანკრეატული ლიპაზის პოტენციური ინჰიბიტორია. ქართულ ბაზარზე არსებული ადგილობრივი ჯიშებისგან (საფერავი და რქაწითელი) დამზადებული ღვინოები ხასიათდება მაღალი ანტილიპაზური და ანტიოქსიდანტური აქტივობით და პოლიფენოლების მაღალი შემცველობით. ღვინის ზომიერი მოხმარება შესაძლებელია სასარგებლო იყოს ჭარბი წონის მენეჯმენტში. კვლევის შედეგებმა შეიძლება გაზარდოს მომხმარებლის ინტერესი და მოთხოვნა კახური ტექნოლოგიით დამზადებული ქართული ღვინოების მიმართ. საჭიროა დამატებითი კვლევების ჩატარება ღვინის ინდივიდუალური ფენოლების ანტი-ლიპაზური აქტივობის შესაფასებლად და ღვინის მიერ ლიპაზას აქტივობის დათრგუნვის *in vivo* შესასწავლად.

კვლევის კავშირი მდგრადი განვითარების მიზნებთან

სიმსუქნე ქრონიკული, მულტიფაქტორული გენეზის დაავადებაა. იგი შეიძლება გამოწვეული იყოს ისეთი მიზეზებით, როგორიცაა ცხოვრების წესი, გარემო პირობები, სოციალური, ფსიქოლოგიური, კულტურული, გენეტიკური,

მეტაბოლური ფაქტორები. ამ კომპლექსურობის გამო არ არსებობს სიმსუქნის პრობლემასთან უშუალოდ დაკავშირებული რომელიმე კონკრეტული მდგრადი განვითარების მიზანი. თუმცა ჩვიდმეტივე მიზანი მოიაზრებს სიმსუქნის პრობლემას და ამ მიზნების მისაღწევად საჭიროა ამ პრობლემის აღმოფხვრა. აქედან გამომდინარე ის მიჩნეულია SDG-ების აჩრდილად.

სიმსუქნის გავრცელება პირდაპირ კავშირშია SDG 3 მიზანთან „ჯანმრთელი ცხოვრება და კეთილდღეობა.“ სიმსუქნე დაავადებაა, ამავდროულად ის სხვა დაავადებების განვითარების რისკსაც წარმოადგენს. არაგადამდები დაავადებების აღმოფხვრა აუცილებელია SDG 3.4 განსახორციელებლად. ექსპერიმენტულმა მონაცემებმა აჩვენა, რომ ღვინოს *in vitro* პირობებში შეუძლია შეასუსტოს PL აქტივობა. შესაბამისად, ის შეიძლება პერსპექტიულ წყაროდ მივიჩნიოთ სიმსუქნის წინააღმდეგ საბრძოლველად.

კვლევის შედეგები ასევე უკავშირდება SDG 2 მიზანს “დაასრულეთ შიმშილი, მიაღწიეთ სასურსათო უსაფრთხოებას და გაუმჯობესებულ კვებას და ხელი შეუწყეთ მდგრადი სოფლის მეურნეობის განვითარებას“. ღვინის ზომიერად მოხმარების სასარგებლო თვისებების შესახებ უამრავი საერთაშორისო კვლევაა გამოქვეყნებული. თუმცა აღნიშნული კვლევები უმეტესად წითელი ღვინის სარგებლიანობაზე მიუთითებენ, მათში პოლიფენოლებისა და ანტიოქსიდანტების მაღალი შემცველობის გამო. ჩვენი კვლევის შედეგები აჩვენებს, რომ თეთრი ქვევრის ღვინოები შეიცავს ევროპული ტექნოლოგიით დამზადებული წითელი ღვინოების პოლიფენოლების თანაზომიერ რაოდენობას. აქედან გამომდინარე, კახური მეთოდით დამზადებული ღვინო შესაძლოა განვიხილოთ როგორც დამხმარე საშუალება სიმსუქნისა და არასწორი კვების გადასაჭრელად.

მიუხედავად იმისა, რომ კვლევის შედეგები ძირითადად დაკავშირებულია SGD 2 და 3-თან, მნიშვნელოვანია აღინიშნოს, რომ საჭიროა სხვადასხვა SDG-ის წარმატებით

განხორციელება სიმსუქნის წინააღმდეგ ღვინის გამოსაყენებლად. ამას გარდა, ზოგიერთი SDG მნიშვნელოვან როლს ასრულებს ღვინის ხარისხის მახასიათებლების განსაზღვრაში. თავდაპირველად გასათვალისწინებელია ინდიკატორი 3.5, რომელიც მიზნად ისახავს „გაძლიერდეს ნარკოტიკების მოხმარების პრევენცია და მკურნალობა, მათ შორის ნარკოტიკული საშუალებების ბოროტად გამოყენება და ალკოჰოლის მავნე გამოყენება“. ეს სამიზნე გულისხმობს, რომ არსებობს ალკოჰოლის მოხმარების ჯანსაღი დონე, მაგრამ ის ასევე აჩვენებს ხარისხიანი განათლების (SDG 4) და პასუხისმგებელი მოხმარების (SDG12) აუცილებლობას. უფრო მეტმა ინფორმაციულობამ შეიძლება გავლენა მოახდინოს სასმელის ჯანსაღ და მდგრად არჩევანზე და პასუხისმგებელ მოხმარებაზე. ასევე მნიშვნელოვანია SDG 10-ის, „შემცირებული უთანასწორობა,“ განხორციელება, რადგან უთანასწორობა იწვევს შემოსავლის, ჯანმრთელობის, სურსათისა და განათლების ხელმისაწვდომობის უთანასწორობას.

მდგრადი წარმოების თვალსაზრისით, საინტერესოა SDG 12. მარნებისა და ღვინის ქარხნების წყლის ხარჯი ძალიან დიდია. აქედან გამომდინარე, მეღვინეობის მდგრადი პროცესისთვის, მნიშვნელოვანია შემდეგი ინდიკატორების განხორციელება :

- ინდიკატორი 12.8 „ნებისმიერ ადგილას მყოფი ადამიანებისთვის შესაბამისი ინფორმაციისა და ცოდნის უზრუნველყოფა მდგრადი განვითარების და ბუნებასთან ჰარმონიული ცხოვრების წესის შესახებ.“
- ინდიკატორი 12.4 - „მიაღწიო ქიმიკატებისა და ყველა ნარჩენის ეკოლოგიურ მენეჯმენტს მათი სასიცოცხლო ციკლის განმავლობაში, საერთაშორისო ჩარჩოების შეთანხმების შესაბამისად და მნიშვნელოვნად შეამცირო მათი გაშვება ჰაერში, წყალსა და ნიადაგში, რათა მინიმუმამდე იქნას დაყვანილლი მათი უარყოფითი ზემოქმედება ადამიანის ჯანმრთელობასა და გარემოზე“

ამას გარდა, ღვინის დამზადებისას საჭიროა სუფთა წყალი (SDG 6). ამ თვალსაზრისით ასევე საინტერესოა ინდიკატორი 6.4, რომელიც მიზნად ისახავს „არსებითად გაზარდოს წყლის გამოყენების ეფექტურობა ყველა სექტორში და უზრუნველყოს მტკნარი წყლის მდგრადი ამოღება და მიწოდება წყლის დეფიციტის აღმოსაფხვრელად და არსებითად შეამციროს წყლის დეფიციტით დაავადებული ადამიანების რაოდენობა“. ეს მიზანი ასევე დაკავშირებულია პასუხისმგებელ წარმოებასთან, რადგან მისი მიღწევა რთულია წყლის გამოყენების ეფექტურობის გაზრდის გარეშე.

ყველა ზემოთ ნახსენები SDG მიზნების გარდა, მნიშვნელოვანია შემდეგი მიზნების წარმატებით განხორციელება ღვინის დახმარებით სიმსუქნის სამართავად.

SDG 1 - „არა სიღარიბე“ – სიღარიბე ზღუდავს წვდომას ადეკვატური სურსათის მიღებაზე და ართულებს კვების რეკომენდაციების მიღწევას. დაბალი შემოსავლის მქონე ადამიანები ფასებზე მგრძნობიარენი არიან, ამიტომ ღვინის მაგივრად სხვა საქონლის შეძენას ამჯობინებენ.

SDG 13. კლიმატის ქმედება - კლიმატის ცვლილება გავლენას ახდენს გლობალური სურსათის წარმოებასა და სურსათის უსაფრთხოებაზე, ასევე სუფთა წყლის რესურსების ხელმისაწვდომობაზე. კლიმატი ყურძნისა და ღვინის წარმოებაში ერთ-ერთი ყველაზე მნიშვნელოვანი ელემენტია, რომელიც გავლენას ახდენს კონკრეტული რეგიონის ყურძნის სპეციფიკური ტიპების ვარგისიანობაზე, ასევე წარმოებული ღვინის ტიპსა და ხარისხზე. ღვინის შემადგენლობაზე დიდ გავლენას ახდენს მეზოკლიმატი და მიკროკლიმატი და მაღალი ხარისხის ღვინოების შესაქმნელად უნდა შენარჩუნდეს კლიმატური-ნიადაგური-ჯიშური წონასწორობა. იმის გამო, რომ ღვინოში ფენოლური ნაერთების არსებობაზე დიდ გავლენას ახდენს ტემპერატურა, საშუალო ტემპერატურის მატება გავლენას მოახდენს მევენახეობის

ზონებში მათ არსებობაზე, შესაბამისად იმოქმედებს ყურძნის ხარისხზე და შემდგომში შეამცირებს ღვინის ანტი-ლიპაზურ აქტივობას.

ზემოთნახსენები კლიმატური-ნიადაგური-ჯიშური წონასწორობა ასევე იწვევს SDG 15-ს (ცხოვრება ხმელეთზე). მიწათსარგებლობის ცვლილება იწვევს ნიადაგის დეგრადაციას, რომელიც თავის მხრივ ამცირებს ბიომრავალფეროვნებას, სურსათის წარმოებას და მტკნარ წყალზე ხელმისაწვდომობას.

საჭიროა ასევე აღინიშნოს SDG 17, „პარტნიორობა მიზნებისთვის“. შესაძლოა საინტერესო იყოს გლობალური პარტნიორობის გაღრმავება ქართულ ღვინის მარნებთან ან ქარხნებთან. მნიშვნელოვანია კახური მეღვინეობის მეთოდით დაყენებული ღვინოების ხელმისაწვდომობის უზრუნველყოფა და მწარმოებლებისთვის ქართული მეღვინეობის ტრადიციის ყველა ეტაპის ახსნა. ბოლოს და ბოლოს, სიმსუქნესთან ერთად ბრძოლა ადვილი იქნებოდა.

სამეცნიერო პროდუქტიულობა

დისერტაციის ექსპერიმენტული მასალების შესახებ სხვადასხვა საერთაშორისო სამეცნიერო ჟურნალში გამოქვეყნებულია შემდეგი სამეცნიერო სტატიები:

- Khatchapuridze Z., Gugulashvili G., Ghvachliani V., Ploeger A., Gulua L., Turmanidze T. (2021). **In-vitro functional efficacy of extracts from Caucasian Rhododendron (Rhododendron Caucasicum) and Rkatsiteli wines as pancreatic lipase inhibitors**, *Ukrainian Food Journal*, 10(1), pp. 37-50.
  DOI: 10.24263/2304974X-2021-10-1-4

- Khatchapuridze Z., Ploeger A., Gulua L., Turmanidze T. (2021). **Comparative study of lipase inhibitory activity of some Georgian wines obtained through Kakhetian and European wine-making techniques**, *Annals of Agrarian Science*, 19(3), pp. 223-234

- Khatchapuridze Z., Ploeger A., Gulua L. **Kinetic Behaviour of Pancreatic Lipase Inhibition by Wine**, *Food Research* **(In Press…).**

კვლევის შედეგები წარდგენილ იქნა მეათე საერთაშორისო სპეციალიზებულ სამეცნიერო და პრაქტიკულ კონფერენციაზე "Trends in LEAN Food Production and Packaging" კიევში, უკრაინაში 2021 წელს.

Abstract

The following study evaluates and compares the anti-lipase activity and bioactive compound contents of fourteen commercially available Georgian wines that differed by the production method (Kakhetian winemaking and Classic European winemaking methods). It also determines an in vitro model of the inhibition mechanisms of chosen wine (Mukuzani) towards pancreatic lipase (PL) by evaluating its kinetic parameters and inhibition mechanism. Additionally, it investigates the inhibitory effect of different phenolic compound fractions of Mukuzani wine against PL and characterises the kinetics of such inhibition.

From the investigated wines, the highest total polyphenol content (TPC) is found in the wine made by Mukuzani Valley 2019 (3572.358 ± 153.111 mg Gallic Acid equivalent (GAE) per litre. This wine also has the highest antioxidant activity (AOA), 4729.199 ± 88.162 mg ascorbic acid equivalent (AAE) L -1 . It is recognised that wines made with the Kakhetian fermentation method contain more total polyphenols than those made by the classical European method. The differences between the samples are statistically significant. White wines made with the Kakhetian method have comparable TPC to some European-style red wines. Red wines, in general, are characterised on average by higher anti-lipase and antioxidant activity than white wines. However, white wine has the highest anti-lipase activity among the investigated samples. 1 mL of the sample is able to inhibit the activity of 1 mg enzyme by 32.63%. Wines from the Mukuzani microzone possess high anti-lipase activity, ranging between 27.12 – 29.78. No significant correlation is found between polyphenol content and anti-lipase activity in wine samples, nor between lipase inhibitory activity and the winemaking method. The mode of inhibition identified for the Mukuzani wine sample is mixed-mode inhibition. In this type of inhibition, the value of K_m (244.329±10.214 μmol·mL^{-1}) is higher, and V_{max} (80875.4±3489.754 μmol·mL $^{-1}$ ·hour $^{-1}$) is lower compared to the value of K_m (170.901±7.544 μmol·mL $^{-1}$) and V_{max} (88735±4036.741 μmol·mL $^{-1}$ ·hour $^{-1}$) for the non-inhibited PL. This inhibitor is able to decrease the PL activity rate and reduce the affinity between the substrate (olive oil) and the enzyme (PL).

The value of inhibition constant K_{i1} is equal to 40.556±1.932, and K i2 was equal to 179.361±8.678 μmol·mL $^{-1}$, meaning that binding affinity to the pancreatic lipase was higher than to the enzyme-substrate complex. Fractionated phenolic compounds of wine un-competitively inhibit PL activity. They are considered mutually nonexclusive inhibitors and a synergistic relationship is found between them. The reduction in the values of apparent V max and K m is observed.

The obtained results allow one to conclude that wines on the Georgian market made from local cultivars, i.e. Saperavi (red) and Rkatsiteli (white), are characterised by noticeably high anti-lipase activity and may play a role in body weight management.

Keywords: Wine, Pancreatic Lipase, Enzyme Inhibition, Kinetic Model.

Acknowledgements

"Keep your dreams alive. Understand that to achieve anything requires faith and belief in yourself, vision, hard work, determination, and dedication. Remember all things are possible for those who believe."

Gail Devers

Three years ago, I commenced one of the most interesting experiences of my life so far. The epigraph above was used for the acknowledgement part of my MSc thesis, and now it is my pleasure to use the same quotation for my dissertation.

I would first like to express my gratitude to the Volkswagen (VW) Foundation (Volkswagen Stiftung) and Shota Rustaveli National Science Foundation of Georgia (SRNSFG) for providing financial assistance and giving me the incredible opportunity to be a PhD student of the joint Doctorate Program "Sustainable Agriculture and Food System'' (SAFS) at the Agricultural University of Georgia.

I would like to express my most sincere thanks to Prof. Levan Gulua, who was always there whenever I ran into a difficulty or had a question about my research or writing. He allowed this paper to be my own work , yet steered me in the right direction whenever he thought I needed it. Thank you, Prof. Levan, for believing in me, my abilities to succeed, my talents, and for your patience. Working with you helped me grow professionally and personally. Thank you for assisting me I continuing the research during the Covid-19 lockdown by driving to and from the laboratory. Joining your lab was one of the best choice I have ever made. There are no regrets but joyful memories in such an amazing environment.

I would also like to offer my special thanks to Prof. Angelika Ploeger. It is my honour to be called one of your PhD students. I appreciate your quick responses, feedback and thoughtful guidance. Moreover, your advice has always been of great help.

I would like to express my deep gratitude to Dr Tamar Turmanidze, another supervisor of my research. Thank you for your enthusiastic encouragement and guidance during the research work.

I wish to thank various people who have helped me academically or professionally. I am particularly grateful to Prof. Teo Urushadze for not only supporting me and believing in me, but also for having a brilliant idea to start this amazing doctoral program together with Prof. Angelika Ploeger. Helpful advices and guidance provided by prof. Nato Kobakhidze are greatly appreciated. I would like to express my very great appreciation to Nino Katcharava, for managing and planning different activities throughout the project, purchasing all the necessary reagents and, in general, for being available when needed. I would like to thank each member of the SAFS family, as they have made my doctoral life a delightful and an unforgettable experience.

I am also grateful to Amiran Zhamurashvili from the winery Mukuzani Valley for donating wine samples.

I must express my very profound gratitude to my family for providing me with unfailing support and continuous encouragement throughout my years of study and through the process of researching and writing this dissertation. This accomplishment would not have been possible without them. Thank you. Last but not least, there has been a big group of people that held my hand when I was feeling exhausted, cheered me up when things did not work as expected, gave me a piece of advice in crucial moments, or simply said the right words at the right time and were there when I most needed them. It is my pleasure to thank all of you. And once again, remember that:

All things are possible for those who believe…

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Abbreviation

AAE – Ascorbic Acid Equivalent

ANOVA - One-Way Analysis of Variance

AOA – Antioxidant Activity

ApoB - apolipoprotein

BL - bile salts

BMI – Body Mass Index

C – Cholesterol

CE - cholesterol esters;

CM - chylomicronsDAG - diacylglycerol

FA - fatty acid

FAO – Food and Agriculture Organization

FDA – Food and Drug Administration

FRAP – Ferric Reducing Antioxidant power

GAE – Gallic Acid Equivalent

LPA - lysophosphatidic acid

MAG - monoacylglycerol;

MG - monoglycerol

MTP - microsomal triglyceride transfer protein

NCD – Noncommunicable Disease

NIH – National Institutes of Health

NIDKK - National Institute of Diabetes and Digestive and Kidney Diseases

OIV – International Organisation of Vine and wine

PL- Pancreatic Lipase

RK1 – Ice wine Satrapezo, Telavi Wine Cellar LTD, 2013

RK2 - Rkatsiteli - Vine Ponto; The Spirit of Georgia LTD, 2016

RK3 - Mr Rkatsiteli from Gurjaani; Mr Wine LTD, 2018

RK4 - Rkatsiteli -Vaziani; Vaziani Company LTD, 2016

RK5 – Rkatsiteli; Kindzmarauli's Marani LTD, 2018

S1- Glekhuri, Khasmi Saperavi; Teliani Valley LTD, 2017

S2 - Matrobela, Saperavi; Matrobela Wines LTD, 2018

S3 - Icewine, Saperavi; Guramishvilis Marani LTD, 2017

S4 -Mukuzani Valley, Mukuzani; Mukuzani Valley LTD, 2016

S5 - Mukuzani Valley, Mukuzani; Mukuzani Valley LTD, 2019

S6 - Rtvelisi, Mukuzani, Rtvelisi LTD, 2018

S7 - Zurab Tsereteli – Mukuzani; Tsereteli Wine and Spirits LTD, 2015

S8 - Zhamurashvili's wine, Mukuzani; Zhamurashvili's wine LTD, 2018

S9 - Nekresi Estate, Mukuzani; Nekresi winery LTD, 2016

SAFS – Sustainable Agriculture and Food Systems

SD – Standard Deviation

TA – Titratable Acidity

Tukey's HSD - Tukey's Honestly Significant Difference

TAG - triacylglycerol

TG – triglycerol

TPC – Total Phenolic Content

UN – United Nations

UV – Ultra Violet

WHO – World Health Organization

1. Introduction

Obesity, which has been reported as a twenty-first-century disease and the "New World Syndrome", is a universal public health concern (Rössner 2002; WHO 2000). Studies have demonstrated a positive relationship between obesity and the intake of a lipid-rich diet (Bray et al., 2004; Schrauwen and Westerterp, 2000). Dietary fats absorption relies on the action of pancreatic lipase (PL) (EC3.1.1.3), thus, the inhibition of this enzyme has become an attractive approach to managing and treating obesity (T.-T. Liu et al. 2020). Taking into consideration that pancreatic lipase is a safe target, and its inhibition does not alter the central pathways, reducing the fat absorption through the inhibition of PL has become the most favourable medical strategy for treating obesity. The only clinically approved pharmacologic agent is Orlistat (Xenical) (Kumar and Chauhan, 2021), which can inhibit the digestion of about 30% of the daily ingested fat. However, the intake of this medicine has been associated with several adverse effects, such as faecal incontinence, stomach pain or discomfort, fatty/oily stools etc. (Cruz-Hernandez et al., 2010; Filippatos et al., 2008; Tak and Lee, 2021). Consequently, continuous efforts are being made to discover and evaluate natural compounds with minimal side effects and inhibitory activity against PL (de la Garza et al., 2011; Kumar and Chauhan, 2021).

A great deal of research showed that the class of polyphenols represents one of the most important sources of inhibiting PL activity (Buchholz and Melzig, 2015; Singh et al., 2020). In this manner, wine is known to be rich in polyphenols (Giovinazzo, Carluccio, and Grieco 2019), and by drinking it, humans consume complex beverages, not single polyphenols. Kakhetian winemaking method makes the wine even richer with polyphenols (A Shalashvili et al. 2007; Armaz Shalashvili et al. 2012). Although extensive research has been done about Georgian wines, by current knowledge, only scarce information about their anti-lipase activity is available (Gulua et al. 2018).

1.2 Research Objectives, Novelty and Scientific Contribution

Research Objectives This research aims to evaluate and compare the anti-lipase activity and bioactive compound contents of commercially available Georgian wines. Because only the inhibition potency itself is not enough to rank new chemicals regarding their inhibitory potency (Brooks et al. 2012), another objective of the present research was to determine the kinetics and mode of inhibition of PL activity by wine by evaluating its kinetic parameters and inhibition mechanism.

Novelty and scientific contribution. To the best of our knowledge, no studies have been conducted to evaluate the kinetic behaviour of PL inhibition by wine.

The practical implication of the study. Findings may play a role in body weight management and benefit in problems associated with excess weight. Additionally, this may increase interest and demand toward Georgian wines made by the Kakhetian technology – a cultural heritage of Georgia. This will be helpful for the economic development of the country.

1.3 The Structure of the Dissertation

The dissertation follows the basic structure and contains five chapters, i.e., introduction, literature review, research methodology, results and discussion, and conclusions. The literature review surveys the problem of obesity and the current strategies used for the treatment and management of obesity. Additionally, it links obesity with Sustainable Development goals (SDGs) and reviews the role of the PL enzyme in obesity. It also reviews natural inhibitors of PL and outlines the perspective of Georgian wine toward this approach. Research methodology gives a clear explanation of design choices by justifying the suitability and reliability of chosen methods and techniques. Obtained experimental data and research findings are discussed in the Results and Discussion chapter. The importance of the

successful implementation of several SDGs is also discussed. Finally, a conclusion is drawn based on the interpretation of the results.

2. Literature review

2.1 Definition, aetiology and classification of obesity and overweight

Overweight and obese individuals have abnormal or excessive fat accumulated, which raises the risk of health problems (Claes, Jeannin, and Braet 2022). The terms "overweight" and "obesity" refer to higher body weight compared to the normal or healthy weight for a given height. Besides, overweight may also be the result of lean body mass (muscle, bone or water)(*NIDDK*, 2022). The regulation of body weight is dependent on the interaction of several factors, such as genetic, environmental and psychosocial, economic, physiologic factors etc. (Jéquier, 2002; Nicolaidis, 2019).

According to the World Health Organization (WHO), the main driver of overweight and obesity is an energy imbalance between energy intake and energy expenditure. Consumed calories that exceed expended calories are the fundamental cause of weight gain (WHO 2000).

National Institutes of Health (NIH) states that obesity is a result of lifestyle choices and poor diet (NIH, 1998). Obesity is often linked with the deficiency of individual micronutrients and is defined as a paradoxical condition of malnutrition (Kobylińska et al. 2022).

NIH and WHO have both adopted the Body Mass Index (BMI) as a practical approach for the clinical assessment of body fatness (NIH 1998; WHO 1997). Although BMI does not measure body fat directly, it has shown a good correlation with it (Gallagher et al. 2000). BMI is a metric that relates body weight to height. It quantifies the degree of fatness or adiposity of an individual based on a ratio of weight in kilograms to height in meters squared. The standard weight status categories linked with BMI ranges for adults are shown in table 1 (CDC 2022)

Table 1: BMI ranges and weight status

BMI (kg/m2)	Weight status	Risk of Poor Health
Below 18.5	Underweight	High

18.5 - 24.99	Healthy	Low
25 – 29.99	Overweight	Low to moderate
30 and above	Obese	
30-34.99	Class One	High
35 – 39.99	Class two	Very high
40 and above	Class three	Extremely high

As seen in Table 1, obesity is subdivided into three categories: Class one, class two and class three. Individuals with a BMI of 30 or above fall into the obese category. A person with a BMI between 25 and 29.99 carries excess weight. In general, a higher BMI increases the risk of health problems.

In accordance with WHO, BMI values are age and gender-independent (WHO, 2000). However, it must be considered a rough guide due to certain limitations. BMI is unable to distinguish between the quantities of lean mass and fat mass. Thus, despite the same BMI index, the degree of fatness can vary in different individuals due to different body proportions. Athletes may have a high BMI without undue health risks (Bhurosy and Jeewon, 2013). In spite of these limits, BMI remains a primary assessment tool due to its convenience, cost-effectiveness, and accessibility in all healthcare settings (Gutin 2018).

2.2 Epidemiology of Obesity and Overweight

Obesity is considered the disease of the twenty-first century (Rössner 2002). Although obesity is preventable, it has almost tripled over the past 46 years (WHO 2021a). Moreover, it is expanding at an alarming rate due to fast food intake (Banik et al. 2020) and low levels of physical activity (Shook et al. 2015). In addition, the economic development of countries shifts the burden of overweight and obesity to the population with lower personal wealth (Templin et al. 2019).

Along with aesthetic problems, obesity causes abnormal physiological metabolism, which leads to a series of physiological (Pi-Sunyer 2002), psychological, and social issues (Sarwer and Polonsky, 2016). According to the WHO (2021), obesity, along with overweight, is linked to more deaths worldwide than underweight. Based on the report, every year, at least 2.8 million people die because of being overweight or obese. However, obesity may be even deadlier than thought. Several studies have associated obesity with the severity of COVID-19 cases and increased fatality in patients (Lockhart and O'Rahilly, 2020; Pettit et al., 2020).

Obesity is regarded as an extremely costly health problem that accounts for 2–6% of total health care costs in developed nations (Bleich et al. 2008)and latest findings shows that it will raise over time (Okunogbe et al. 2021). Concerning the prevalence and mortality of chronic metabolic diseases, treatment and management of excessive adiposity remain a challenging endeavour.

2.3 Obesity and Sustainable Development Goals

2.3.1 Sustainable development goals

In 2015, the United Nations General Assembly set the Sustainable development goals (SDGs), which are intended to be achieved by the year of 2030. SDGs consist of 17 goals and 169 targets and aim to transform our world by tackling multiple challenges faced by humankind. SDGs aim to ensure well-being, economic prosperity, and environmental protection. All United Nations member states have agreed to work towards this and set a vision for a world free from poverty, hunger and disease (UN 2019).

The agenda was built on the Millennium Development Goals (MDGs), signed in September 2000 by the UN Member States. There were eight goals set to achieve targets to combat poverty, hunger, disease, illiteracy, environmental degradation and discrimination against women by 2015 (UN 2022). The SDGs aim to be relevant to all countries, i.e., rich, poor and middle-income, to promote prosperity while protecting the environment and tackling

climate change. They have a strong focus on improving equity to meet the needs of women, children, and disadvantaged populations in particular so that "no one is left behind" (UN 2019).

2.3.2 The link between SDGs and obesity

As discussed above, obesity is a global public health concern yet not featured in any SDGs. Overall, many factors can cause obesity/overweight, such as food, health (genetics), financial resources, education, environment, equality, and means of implementation (Qasim et al. 2018). Due to this complexity, no specific goal nor target exists for excessive adiposity. However, it is hidden in plain sight and is incorporated throughout the SDGs. SDGs with the most obvious connection to obesity are SDG 2 and SDG 3. Research has highlighted multiple points at which obesity is affected by the goals (Lobstein and Cooper, 2020; Ralston et al., 2021). Lobstein and Cooper (2020), highlighted 14 relevant goals addressing obesity (see Picture 1) and considered obesity a ghost of SDGs.

2.3.2.1 Obesity and SDG 3 (Ensure Healthy Lives and Promote Wellbeing for All at All Ages)

Several targets under SDG 3 are directly linked to obesity. This goal strives to encourage well-being and guarantee healthy lives at all ages, which is crucial to fulfilling all other economic, environmental and social goals (World Obesity Federation 2021). Target 3.4 calls for reducing mortality from non-communicable diseases (NCDs). Obesity is a chronic disease, which on the other hand, is the root of other NCDs. It is an important risk factor for many severe diseases and health conditions (WHO 2000), such as for example high blood pressure (hypertension) (Aronow 2017; Pausova 2006), high LDL cholesterol, high levels of triglycerides (dyslipidemia) or low HDL cholesterol (Feingold 2020; Franssen et al. 2011), coronary heart disease and stroke (Ortega, Lavie, and Blair 2016), type 2 diabetes (Hossain, Kawar, and El Nahas 2007), Gallbladder disease (Dittrick et al. 2005), osteoarthritis (Pottie et al. 2006), sleep apnoea and breathing problems (Piper and Grunstein, 2010; Resta et al.,

2001), some cancers (Bhaskaran et al., 2014; Calle and Kaaks, 2004; Renehan et al., 2008) and cancer-related mortality (Calle et al. 2003; Reeves et al. 2007), etc.

Concerning the prevalence and mortality of chronic metabolic diseases, treatment and management of obesity remain a challenging endeavour to achieve universal health coverage (SDG target 3.8). Several studies have highlighted a correlation between air pollution and obesity (An et al. 2018; Z. Yang et al. 2019). Thus, it connects obesity to target 3.9, which aims to reduce death and illness from air pollution.

2.3.2.2 Obesity and SDG 2 (End Hunger, Achieve Food Security and Improved Nutrition and Promote Sustainable Agriculture)

Ireland has voluntarily recognised obesity as an intrinsic aspect of SDG 2 ('Ireland: Voluntary National Review' 2018) .

SDG target 2.2 aims to end all forms of malnutrition. As noted in section 2.1. obesity is associated with the nutrition and qualitative malnutrition of the body. Additionally, targets 2.4 and 2.b are connected to health. Target 2.4 implies a connection between climate change and health, and 2.b can be implemented to leverage the elimination of subsidies on unhealthy products.

2.3.2.3 Obesity and SDG 1 (End Poverty in All Its Forms Everywhere)

The term "poverty-obesity paradox" is widely used to describe the positive connection between deprivation and obesity. Although income has not been associated with obesity in most countries (Zhou 2021), studies have outlined the link between obesity and poverty across the United States (Bentley, Ormerod, and Ruck 2018) and the United Kingdom (Holmes 2021). The high risk of obesity among low-income individuals is due to the affordable prices of unhealthy food and beverages compared to healthy alternatives.

Therefore, halving poverty (SDG target 1.2) will have a positive impact on reducing obesity. Additionally, taxing foods with high content of fat, sugar and salt and removing a tax on vegetables and fruit are likely to reduce inequities.

2.3.2.4 Obesity and other relevant SDGs

Other Goals that are related to obesity are SDG6 (addressing access to safe water), SDG 12 (addressing sustainable consumption and production) and SDG 15 (addressing the use of land). These goals are connected to healthy and sustainable diets, thus impacting obesity and overweight.

Poor accessibility to safe drinking water (target 6.1) may lead to the consumption of bottled beverages, including sugary drinks, as they are the safest option. The intake of soft drinks is associated with a greater risk of developing overweight (Luger et al. 2017; WHO 2017).

Excessive consumption and production of food have been considered to have a negative impact on human health and the planet (Willett et al. 2019). Dietary overconsumption causes a positive energy balance, leading to weight gain (Fay, Finlayson, and King 2013).

Overall, planetary health is closely tied to human health. Target 15.2 calls to reduce deforestation by half. The majority of global deforestation is nutrition-related and caused by cattle ranching (FAO 2020). A systematic review by Schlesinger et al. (2019), and mediation analysis by Kim et al. (2022), have suggested that consumption of red meat and processed meat is associated with a greater risk of obesity and overweight.

Additionally, SDG 14 is calling for urgent action against climate change. The implementation of this goal is crucial for the sustainability of food systems to avoid reducing healthy diet

options and losing the nutritional content of food crops (Willett et al. 2019). Furthermore, the rise in global temperature may have a negative impact on physical activity, subsequently increasing the risk of overweight or obesity (Koch et al. 2021).

It is also worth noting that a clean environment has an important impact on the healthy living of human beings (Nicolaidis 2019). Target 11.3 aims to increase '*inclusive and sustainable urbanisation*'. Besides, target 11.7 calls for access to public green spaces. Physical activity is an essential driver for preventing and managing obesity, and it is a more attractive option in less polluted places. Additionally, according to Parasin et al. (2021), increased air pollution prevails overweight and obesity in children.

Goal related to quality education (SDG 4) offers a solution for preventing obesity and overweight. Target 4.1 calls for free primary and secondary education with effective attainments. This target is especially important for girls since, in many cultures, housewives are in charge of cooking and responsible for the health of the children. Besides, implementation of target 4.6 (on literacy throughout life) will positively decrease the vulnerability of risk factors for overweight and obesity. This target will raise awareness about treatment options for overweight and obesity. It will also help adults to read the label and understand nutrition guidance. According to (Storcksdieck genannt Bonsmann and Wills, 2012), educational campaigns on food labelling are required to prevent excess weight gain.

In addition to education, reducing gender inequity (SDG 5) and inequalities (SDG 10) are essential to encourage overweight and obese individuals. Both SDGs are highly relevant to issues of stigma and discrimination against individuals with high BMI. Target 5.1 implies ending all forms of discrimination against young and adult females. Many studies suggest that women experience weight-based discrimination at higher rates than men. With a meta-analytic examination, Vanhove and Gordon (2014), showed that obese women face discrimination in the workplace more often than male peers. Furthermore, poor

socioeconomic status and societal inequality are linked to obesity (Watts et al. 2016). Flint et al. (2016) showed that obese candidates were judged significantly less suitable in the recruitment process than normal-weight peers. Besides the workplace and recruitment process, weight-based discrimination and bullying remain permitted almost everywhere (Puhl 2022). To tackle this form of inequality, targets 10.2 (on inclusion) and 10.3 (equal opportunities) should be actioned.

Last but not least, the implementation of the following targets out of SDG 17 is needed to maximise the prevention and management of overweight and obesity. Within the frame of target 17.1 (domestic resource mobilisation), health-related tax relief and/or taxation on products can be established. Taxation on sugar-based beverages is a practical approach to lower daily sugar intake. Currently, over 50 countries have implemented taxes on sugary drinks (Obesity Evidence Hub n.d.).

Target 17.3 (on additional resources for developing countries) - Developing countries are poorly prepared to manage obesity prevalence and the NCDs crisis (Muzammil and Lopes, 2021). Extra resources for developing countries, i.e., funding and innovation, are needed to support health systems.

Target 17.17 (on multisector partnership) is an excellent opportunity to reduce adiposity. Even though obesity is a multifactorial disease, it is highly preventable. Hence broader approaches and cross-sectoral collaboration with the engagement of multiple partners (i.e., private sector, government and civil society) are required to maximise the prevention and management of overweight and obesity (WHO 2007).

Figure 1. Schematic representation of SDGs associated with obesity

2.4 Management of obesity

The increasing rate of worldwide obesity requires prevention and management strategies to alleviate the future health and economic cost of this concern. Numerous strategies have been tested to prevent the increase of adipose tissues (Apovian et al. 2015; Flodmark, Marcus, and Britton 2006); however, these attempts have had little effect up to now (WHO 2021b).

When prevention of obesity fails, treatment is advised (George A Bray et al. 2016). Several guidelines have been introduced by different countries, e.g. UK (National Clinical Guideline Centre (UK) 2014), USA (VA/DoD 2020), and Canada (Wharton et al. 2020). Overall

guidelines for European countries have also been initiated (Durrer Schutz et al. 2019; Yumuk et al. 2015). Assessment of the degree of adiposity and management of body weight are two main steps in the treatment process (North American Association for the Study of Obesity et al. 2000). Management includes the use of surgeries (Albaugh and Abumrad, 2018; Angrisani et al., 2017), drugs (Apovian et al. 2015) and/or lifestyle changes (Wadden et al. 2006).

Surgery is a powerful approach with long-lasting efficacy for treating clinically severe obesity (Chang et al. 2014). However, it is BMI and age-specific. It is recommended for individuals with a BMI ≥ 40 kg/m^2. Patients with serious comorbid conditions, having BMI between 35.0 and 39.99 kg/m^2, are also eligible for bariatric surgeries (Buchwald et al. 2009). Additionally, the cost of surgery and after-surgery care is high (N. Shah et al. 2016). Moreover, it is also linked with the risk of acute kidney injury (Espino-Grosso and Canales, 2017), increased incidence of marginal ulcers, bowel obstructions, gall stones and nutritional deficiencies (Gasmi et al., 2022; Ma and Madura, 2015).

Lifestyle modifications, i.e. dietary interventions, physical activity interventions and behavioural modification, are primary approaches to weight management. This program can help overweight and obese individuals to lose 5-10% of their initial weight. Regular physical activity along with healthy eating habits, a balanced diet and behaviour strategies is known as the most powerful intervention (Salam et al. 2020). Nevertheless, lifestyle intervention is associated with long-term weight loss (Wadden et al. 2020). Additionally, studies have shown that maintaining a weight loss is an issue for more than half of patients (Hall and Kahan, 2018; Wadden et al., 2012).

To achieve effective weight loss and maintain results, most frequently, a combination of long-term lifestyle changes with short-term pharmacologic treatment is (Bays et al. 2022; McCafferty, Hill, and Gunn 2020). With a randomised trial, Wadden et al. (2005), demonstrated the effectiveness of the combination of weight-loss drugs and lifestyle

modification. Study results indicated that the combination of these two strategies improves the weight than any of these alone. Nowadays, most of the guidelines on weight management recommends medical treatment through lifestyle modification (Apovian et al. 2015; Seo et al. 2019).

Weight-loss medications are prescribed by health care professionals for adults with a BMI of 30 kg/m^2 or higher or patients having a BMI of 27 or greater with a comorbid condition of obesity (such as high blood pressure, type 2 diabetes etc.). Pharmacotherapy is only allowed with lifestyle intervention since the intake of anti-obesity drugs solely is not enough for weight loss (Tak and Lee, 2021).

Different approaches and mechanisms of actions are being used to develop weight loss therapeutics (Seoane et al. 2014). Weight management medications work in different ways and can either act centrally or peripherally (Bays et al. 2022). Drugs that act on the central nervous system regulate food intake through the sensation of fullness and satiety. Usually, these medications affect the regulation of energy homeostasis (Yeung and Tadi, 2022). Peripherally acting therapeutics influence lipogenesis, metabolism and digestion. More specifically, peripheral modulators can affect the absorption of dietary fat or carbohydrates, metabolism and storage of fat, or elevation of heat production from dietary fat (Seoane et al., 2014).

2.5 The Role of Lipase in Obesity

Apart from the genetic factors, in modern society, the intake of high-fat foods has been associated with obesity (Bray and Popkin, 1998; Schrauwen and Saris, 2006). One gram of fat provides nine kcalories, which is more than twice as the one of protein and carbohydrates, consequently making food energy-dense. As discussed in section 2.1, energy imbalance is the main driver of obesity. Lack of physical activities and increased intake of junk foods lead to

adiposity. Thus, the development of adiposity has been linked to the metabolism of lipids (Kayode 2021).

The main constituent (90%) of dietary fats are mixed triacylglycerols (**TAGs**), also referred to as triglycerides (TGs) (Lichtenstein 2013). TAGs are composed of three molecules of fatty acids and one molecule of glycerol. Before absorption in the gastrointestinal tract, TAGs undergo a series of biochemical reactions. Additionally, the breakdown of TAGs is important to facilitate the assimilation of dietary fat into the body. Two different types of lipases, pre-duodenal (i.e. lingual and gastric) and extra-duodenal (i.e. pancreatic), are involved in the digestive system. Hepatic, lipoprotein and endothelial lipases are also presented in the small intestine.

Lipases are also known as triacylglycerol hydrolases (EC.3.1.1.3) (Brenda, 2022) and belong to the family of carboxylic ester hydrolases. They are generally defined as monomeric and water-soluble enzymes that catalyse the hydrolysis of ester substrates (Brockman 2004). The process of digestion and absorption of dietary lipid is given in Fig.1

The pathway of dietary lipid digestion starts in the stomach, where **TGs** are partially hydrolysed by lingual and gastric lipases. The degradation of fat by the lingual lipase is very small due to the acidic environment. However, in the case of infants and young children, around 50-70% of the dietary fats can be degraded. On the other hand, gastric lipase is active and stable at an acidic pH. Gastric lipase partially hydrolyses dietary fat (10-30%) to diacylglycerol and free fatty acids (FAs) and forms large fat droplets (with hydrophobic TAG cores). These globules are encircled by polar molecules, such as fatty acids (**FAs**), cholesterol (CL), ionised proteins and phospholipids (PLs). Gastric lipase is often considered as an important factor to regulate PL secretion (T.-T. Liu et al. 2020).

Pancreatic lipase is synthesised and secreted in the pancreas and is the main lipolytic enzyme. It plays a crucial role in the efficient digestion of triglycerides and accounts for 50-

70% of dietary fat hydrolysis. Digestion of dietary fat is completed in the small intestine, where fat globules are mixed with PL and bile salts (BS) to form an aqueous suspension of tiny fatty droplets. To add an acyl group, PL forms an enzyme-substrate complex by anchoring at the interface of fat micelle. Acetylated PL is able to accumulate with other native enzymes. However, bile acids inhibit the lipolytic activity of PL; Inhibition is overcome by pancreatic colipase, which acts as a cofactor, binds to the non-catalytic domain of lipase and forms a stoichiometric complex. It also binds to the bile-salt covered triacylglycerol interface and consequently allows the enzyme to anchor itself to the water-lipid interface. Colipase, as a precursor molecule, is produced by the exocrine pancreas. PL hydrolysis TAGs to monoacylglycerol (MAG) and free FAs. The products of lipid hydrolysis together with lysophosphatidic acid (LPA), CL, BS and fat-soluble vitamins mixed micelles. These micelles are the source of digested dietary products, which are engrossed by enterocytes. MAG, FAs, and CL can cross the brush-border membranes of enterocytes through passive diffusion. Absorbed MAG is then resynthesised to form TAG, whereas dietary CL is acylated to cholesterol esters (CE). TAG joins CE and apolipoprotein B (ApoB) with the help of microsomal triglyceride transfer protein (MTP) and forms chylomicrons (CM). CMs enter circulation through the lymph. An increasing number of CM affects obesity. TAGs are also stored in adipocytes as their main energy source. Excessive accumulation of TAGs in adipose tissue causes obesity (Shi and Burn, 2004).

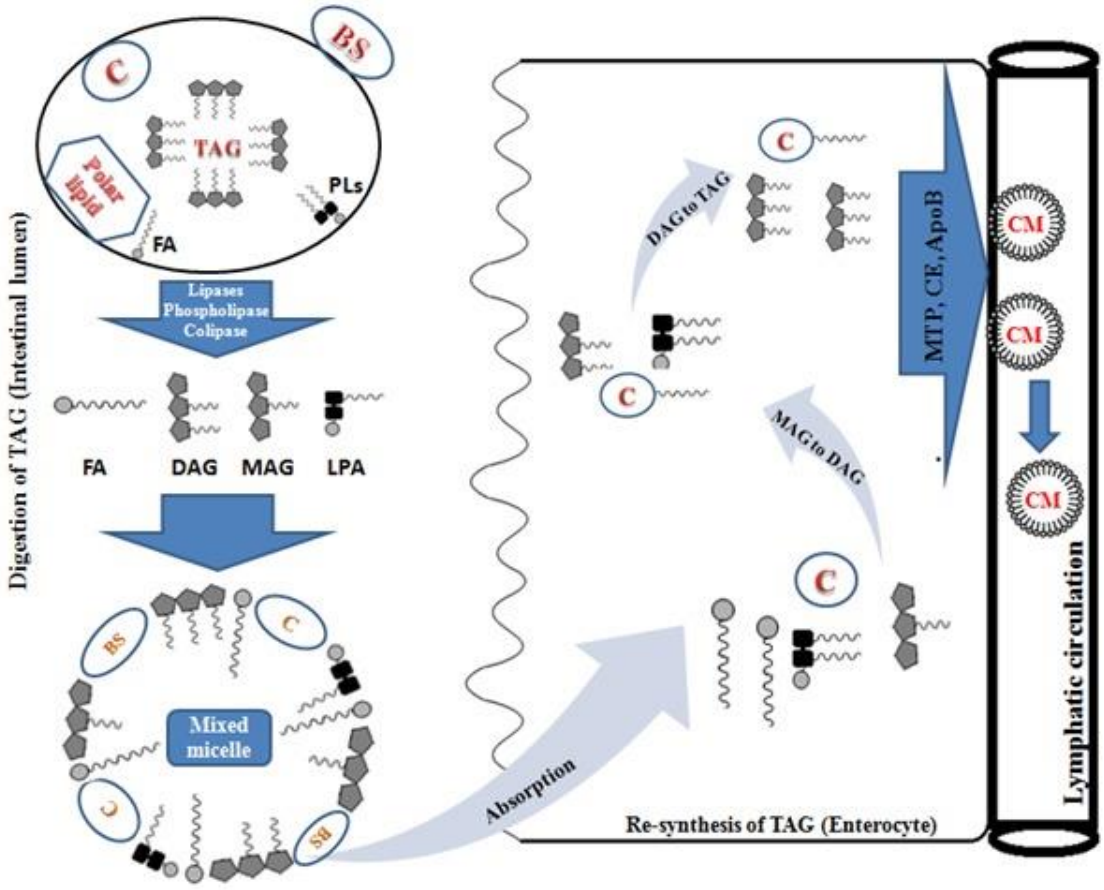

Figure 2. The Process of Digestion and Absorption of Dietary Lipids; FA: fatty acids; TAG: triacylglycerol; DAG: diacylglycerol; MAG: monoacylglycerol; PLs: phospholipids; C: cholesterol; BS: bile salts; LPA: lysophosphatidic acid; MTP: microsomal triglyceride transfer protein; CE: cholesterol esters; ApoB: apolipoprotein B; CM: chylomicrons

Additionally, a schematic illustration of the molecular mechanism and biological function of PL provided by Kumar and Chauhan (2021), is shown in Fig. 2. Understanding the path of an enzyme-catalysed reaction is important to identify or develop different types of inhibitors with the desired mechanism of action (Holdgate, Meek, and Grimley 2018).

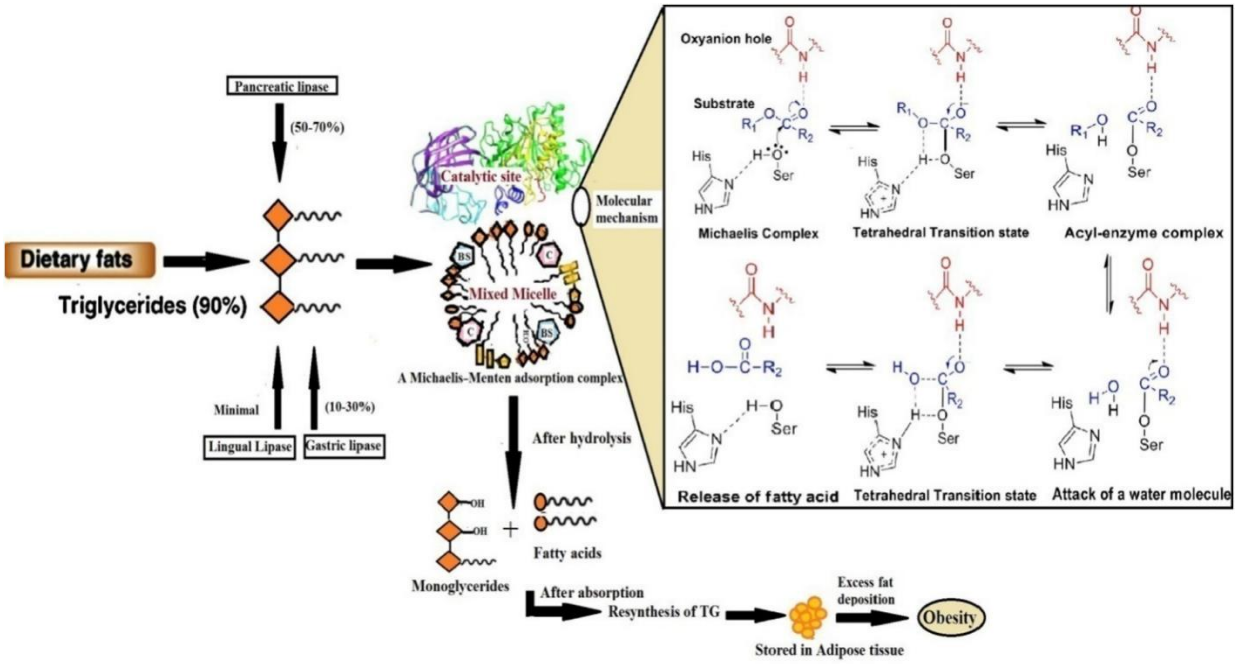

Figure 3. The Mechanistic Approach of PL for Digestion of Fat –Pathway in The Human Body.
C-cholesterol, BL – bile salts; FA: fatty acids; MG: monoglycerol; TG: triglycerol; LPA: lysophosphatidic acid;

2.6 Inhibition of Pancreatic Lipase in Obesity Management

Using lipase inhibitors to reduce energy intake represents an important and common treatment strategy for obesity and overweight (de la Garza et al. 2011; Seyedan et al. 2015). As discussed in section 2.4. weight loss medications are divided into two categories. Lipase inhibitors are peripherally acting anti-obesity drugs. Compared to the centrally acting drugs, lipase inhibitors have been proven to be relatively safe. The main advantage of these types of inhibitors is that they do not enter the nervous system or human blood vessels. Furthermore, no effect on the mineral balance and bone circulation has been observed. On the other hand, several drugs that act on the central nervous system have been found to cause adverse cardiovascular or mental reactions. Consequently, they have been withdrawn, which also increased the interest in the inhibition of PL activity.

The inhibition of PL is the most widely studied mechanism for the identification of potential anti-obesity agents. PL inhibitors are reactants that slow down or stop catalysis in the small

intestine and, consequently, decrease the absorption of fat. Overall, the inhibition caused by the drug can be either reversible or irreversible. An irreversible PL inhibitor forms a stable complex and covalent bond with the enzyme. As a result, PL is either permanently inactivated or slowly reactivated.

A reversible PL inhibitor is a substance that slows down, or inhibits, the reaction rate. Compared to irreversible inhibition, reversible enzyme inhibition involves a non-covalent bonding. Physiological inhibitors that control metabolic pathways and synthetic inhibitors are part of this group. Three types of reversible inhibition exist such as uncompetitive, competitive, and non-competitive. These also include mixed inhibitors (competitive-uncompetitive and competitive-non-competitive) (Garrett and Grisham, 1999)). Reversible inhibitors can be therapeutically valuable. Therefore, the work by a variety of mechanisms needs to be distinguished by observing the kinetics of enzyme inhibition.

Competitive inhibition occurs when a drug similar to PL is present and competes with the substrate for the active site on the enzyme. Alternatively, a non-competitive inhibitor does not compete with the substrate for the active site and binds to the allosteric site on the enzyme. The uncompetitive inhibitor is capable of binding either to the free enzyme or the enzyme-substrate complex. Uncompetitive inhibition happens at high concentrations of the substrate since the inhibitor only binds to the enzyme-substrate complex (Voet and Voet, 2011).

Fig. 3 is the illustration of the lipid metabolic pathway in the human body in the presence and absence of inhibitors. The lipase inhibitors, by combining with the active lipase part of the stomach and small intestine, changes the conformation of the stomach/trypsin, inhibit catalytic activity, and thus reduce lipids such as triglycerides. Hydrolysis reduces the digestion and absorption of lipids in food as well as the accumulation of adipose tissue and achieves the effect of controlling and treating obesity. After the lipase inhibitor acts, it is

usually excreted along with the lipase to which it is bound. Therefore, it does not cause long-term effects on the human body.

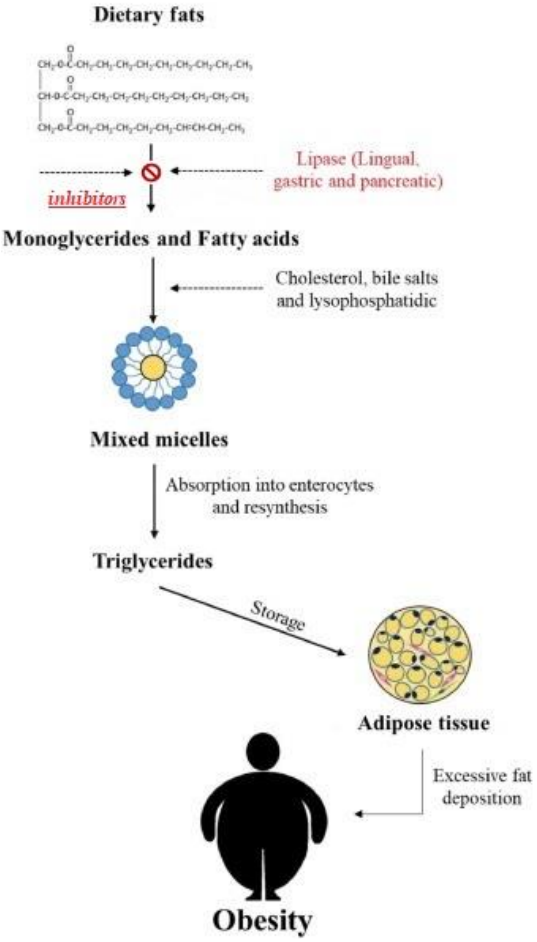

Figure 4. Lipid metabolic pathway in the human body

2.7 Orlistat as a Pancreatic Lipase Inhibitor

Orlistat® is the only clinically approved drug used to reduce intestinal fat absorption. It is a modified bacterial drug, a hydrogenated derivative of lipstatin, isolated from the Gram-positive bacterium Streptomyces toxytricini (Bogarin and Chanoine, 2009). The principal mechanism of Orlistat involves the inactivation of gastrointestinal lipase, consequently preventing the absorption of dietary fats. This reversible inhibitor results in the reduction of dietary fats by about thirty per cent (Müller and Geisler, 2017)

Orlistat is a competitive inhibitor of PL (Birari and Bhutani, 2007); it covalently binds to the serine residues of active sites of lipases and inactivates them. The inactivation of lipases prevents the hydrolysis of triglycerides, and thus free fatty acids are not absorbed.

Orlistat is a natural inhibitor of PL and was first approved by the FDA in 1999 (The Center for Drug Evaluation and Research 2018). In Europe, 120 mg capsules of Orlistat are available under the name Xenical, whereas the 60 mg formulation goes under the brand name Alli. A dose of 120 mg Orlistat is available on prescription only, while 60 mg of the drug is approved for over-the-counter sales, although double dosing of Alli is not recommended.

Orlistat has been used for long-term management and treatment; however, it has to be used as a part of a weight-loss plan. Additionally, there are some critical considerations before starting the intake of the drug.

Orlistat is only prescribed to those with a BMI of at least 30 or individuals with a BMI of 28 or greater, also suffering from other weight-related conditions. Additionally, taking the drug is not recommended during pregnancy and lactation. Orlistat can only be used when no more than 30% of the calories come from fat (Buse JB, et al., 2009). Dietary reference intake (DRI) for fat in the adolescent age group ranges between 20-35% and varies between 44 and 77 grams when on a 2 000 calories diet. The clinical dose of Orlistat is 120 mg. It can be taken three times a day, with liquids during or after ingestion of fatty foods. In case the meal does not contain fat or is missed, Orlistat should be omitted. While on the treatment, patients are expected to lose 5% of the starting body weight in three months. If this is not the issue, the use of Orlistat should be terminated.

Orlistat has a profitable drug-interaction profile since it is minimally absorbed. It is barely metabolised; approximately 83% of the extracted drug is intact Orlistat. Studies have shown that the accumulation of Orlistat is minimal in both short and long-run periods (Henness and

Perry, 2006).According to the trials, Orlistat is more effective than diet alone for weight reduction and maintenance of lost weight. Its long-term administration is associated with a small but statistically significant weight loss of about 3% more than diet alone in overweight and obese people (Drew, Dixon, and Dixon 2007). Additionally, Orlistat treatment results in modest improvements in total cholesterol, low-density lipoprotein, blood pressure, and fasting glucose and insulin concentrations. Moreover, it also decreases blood pressure, prohibits the onset of DM type II and improves oral glucose tolerance (Seyedan et al. 2015).

Orlistat, compared to appetite-suppressing drugs, is free of serious side effects. However, the ingestion of this compound is still associated with a number of adverse effects. The most common adverse effects are gastrointestinal, i.e., liquid stools, diarrhoea, steatorrhea, bloating, faecal urgency, etc., mainly caused due to the impaired absorption of dietary fat (Cruz-Hernandez et al. 2010; Filippatos et al. 2008). Although these side effects can be minimised by following a low-fat diet, they are often unacceptable to patients (Bansal and Khalili, 2022).

Additionally, Orlistat may decrease the absorption of fat-soluble vitamins (A, D, E, K). Thus, during the therapy, daily intake of multiple-vitamin supplements is recommended (Heck, Yanovski, and Calis 2000). Moreover, Humayun et al. (2016) showed that Orlistat could increase the risk of acute kidney injury. In the intestinal lumen, Calcium preferentially binds to unabsorbed fat, instead of the oxalate. Oxalate is easily reabsorbed, which leads to oxalate nephropathy and an increased risk of renal stones. Because of the impaired absorption of calcium and vitamin D, Orlistat theoretically can increase the risk of osteoporosis (Bansal and Khalili, 2022).

Due to the adverse effects of Orlistat stated above, there has been an increased interest in discovering other natural substances with potent PL inhibitory activity and fewer side effects.

2.8 Targeting Obesity with Natural Lipase Inhibitors

The success of Orlistat has evoked interest in the identification of newer PL inhibitors that lack some of these unpleasant side effects. Nowadays, experimental screening of natural products or chemical synthesis uses the inhibitory activity of Orlistat as a reference for comparative study.

Numerous plant metabolites have been screened for the purpose of discovering naturally occurring potent lipase inhibitors. Plant-derived PL inhibitors include proteins like those found in Litchi chinensis (Mhatre, Bhagit, and Yadav 2019) and Seabuckthorn seed meal (Xiang et al. 2020). Other plant origin lipase inhibitors are polysaccharides. Li et al. (2021) showed that PL activity may be inhibited by mulberry leaf polysaccharides in-vitro and in vivo. Additionally, the gastrointestinal side effects of mulberry leaf polysaccharides were similar to the one of Orlistat. However, the study highlights the importance of further investigations into the mechanisms of mulberry leaf polysaccharides prevention and treatment of obesity is still needed.

Using porcine PL and p-nitrophenyl butyrate in an in vitro experiment, Lai et al. (2014) assessed the lipase inhibitory activity of methanolic extracts of different medicinal plants. Overall, they screened different parts of 32 plants, located in Malaysia. Twenty-seven crude extracts out of thirty-two revealed inhibitory action against porcine PL in vitro. Eleusine indica had the highest inhibitory impact on PL and inhibited its activity by 31.38 ± 0.58%. Orlistat itself inhibited PL activity by 34.49 ±5.39%. In contrast, the inhibitory efficacy displayed by 19 crude extracts against PL was only 10%.

Lipase inhibitory potential has also been reported by various phytochemicals, such as alkaloids (Wikiera, Mika, and Zyla 2012), Carotenoids (Matsumoto et al., 2010; Wan-Loy and

Siew-Moi, 2016), Saponins (R. Liu and Xu, 2015; Navarro Del Hierro et al., 2021), and terpenes (Lee et al. 2005; Sheng et al. 2006), etc.

It is worth mentioning that a deeper understanding of molecules, such as isolation, identification and characterisation, is required to discover powerful, potent PL inhibitors and to evaluate synergistic effects, with the numerous natures of the compounds that complicate the outcomes. For this purpose, recent approaches have started to utilise in-silico computational modelling methods. These approaches aim to find out the interaction of Phyto-molecules with specific active binding site of pancreatic lipase (Rajan, Palaniswamy, and Mohankumar 2020).

For this purpose, Yang et al. (2021) developed a rapid screening approach to identify natural lipase inhibitors from *Alisma orientale*. The method combined mass spectrometry with high-performance thin-layer chromatography-bioautography and discovered eleven lipase inhibitors from the extracts of the plant. These compounds were associated with triterpenoids by combining the spectral and chromatographic features with the data from the literature.

2.8.1 Polyphenols as Pancreatic Lipase Inhibitors

Polyphenols represent the major class for the PL inhibitor. They bind to the enzyme by polyvalent sites present in them (Lunagariya et al. 2014). A great deal of research showed that the class of polyphenols represents one of the most important sources of potential PL inhibitors (T. Buchholz and Melzig, 2015; Martinez-Gonzalez et al., 2017). PL inhibition has been reported by numerous polyphenolic compound-rich foodstuffs, including medicinal plants (Seyedan et al. 2015; Zheng et al. 2010), berries (McDougall, Kulkarni, and Stewart 2009; Sosnowska, Podsędek, and Kucharska 2022), cocoa (Gu et al. 2011), tea (Glisan et al. 2017; Gondoin et al. 2010), grape seeds (D. A. Moreno et al. 2003; Serea et al. 2022), etc.

Phenolic substances may be useful in avoiding obesity by reducing the activity of fat-metabolizing enzymes such as PL, lipoprotein lipase, and glycerophosphate dehydrogenase (Yoshikawa et al. 2002). Over 8000 polyphenolic chemicals of various structures and functions have been specified in the plant kingdom (Dai and Mumper, 2010). The lipase inhibitory capacity has been documented for more than 70 different flavonoids, and the inhibitory effect depends on the number and position of phenolic hydroxyl groups (T. Buchholz and Melzig, 2015). Flavonoids and phenolic acids are two widely consumed (González-Castejón and Rodriguez-Casado, 2011) and probably the most studied chemical classes of phenolics showing anti-lipase activity (T. Buchholz and Melzig, 2015). Besides the chemical structure, the interaction of polyphenols with other chemicals, as well as the dose employed for treatment or intervention, have a significant impact on their bioavailability and bioactivity (D'Archivio et al. 2007).

In a recently published review article, Singh et al based on the published data, concluded that natural polyphenols could be effectively incorporated to manage obesity.

Flavonoids are a type of plant metabolite with a large number of polyphenolic grouping structures that are abundant in plants (Spencer). They are subdivided into different subclass, i.e. anthocyanidins, dihydroflavanoles, flavanols, flavones, flavanones, bi-and isoflavonoids (Bravo 2009). Klaus et al. (2005) examined the effect of epigallocatechin gallate in mice for a period of 11 months and observed a significant reduction in body weight. Although this flavonoid promoted fat oxidation, fat-reducing effect was entirely explained by its effect in reducing diet digestibility. Epigallocatechin gallate was purified from green tea (*Camellia sinensis*).

Overall, many herbal teas have been vastly studied for the PL inhibition due to the presence of polyphenols. From this point of view, Oolong tea plant contains high levels of

polyphenols. An aqueous decoction of the tea plant is widely used as refreshment drink, apart from traditional medicinal use. Apart from its traditional medical value, an aqueous infusion of the tea plant is frequently consumed as a refreshing beverage. In vitro inhibition of PL by Oolong tea polyphenols was studied by Nakai et al. in 2005. Total of fifty-four polyphenols were isolated from tea leaves extracted and their inhibitory potential were evaluated. One of the primary polyphenols in green tea, (-)-Epigallocatechin 3-O-gallate, inhibited lipase activity with an IC50 of 0.349 μM. (-)-epigallocatechin-3,5-digallate that belongs to the flavan-3-ol digallate esters was able to inhibit PL more effectively, with an IC50 of 0.098 μM.

Nagao et al. (2009)conducted in vivo research to evaluate the role of green tea in obesity. Study was double-blind trial with a duration of 12-week. Participants were treated with catechin rich beverage containing, containing 582.58 mg catechin. Control group was ingested 96.3 mg of catechins per day. The results of the study indicated that catechin rich beverages could be useful for the prevention of obesity. In another trial, Nagao and his colleagues administered were treating participants with a bottle of Oolong tea. The tea contained 690 mg green tea catechins, whilst the drink for control group was containing 22 mg. According to the findings of the study, body weight, BMI, and waist circumference were decreased significantly (Nagao et al. 2005).

Rivera et al. (2008) examined the effects of quercetin at 2 or 10 mg/kg in obese *zucker rats* for 10 weeks, and the results revealed a significant reduction in body weight in both lean and obese rats. Additionally, Kobori et al. (2009) studied the effects of quercetin in male C57/BL6 J mice at different concentration, ranging between of 0.1 and 0.5 %. The duration of study was 2 weeks and results showed significant reduction of body weight, hepatic and visceral and hepatic fat in mice.

Marrelli et al. (2019) assessed lipase inhibitory activity of aerial parts from *Leopoldia comosa* (L.) Parl. Within the study different fractions of extracts (i.e., ethyl acetate, dichloromethane and n-hexane) were obtained and their PL inhibitory potency was compared with orlistat. Results showed that n-hexane and acetone fractions have potent PL inhibition with an IC50 value of 0.369 ± 0.020 and 0.336 ± 0.007. The raw extract and dichloromethane fraction showed less PLE inhibitory action, 3.819 ± 0.119 and 1.409 ± 0.033 respectively, whereas the inhibition value of orlistat was equal to 0.018 ± 0.001. The lipase inhibitory activities exerted by the fractions were linked to presence of different phenolic acids. For example, PL inhibitory activity observed in dichloromethane fraction was related to the presence of ferulic and p-coumaric acids, while the inhibitory action of ethyl acetate fraction was correlated with the existence of the flavonoid glycoside rutin.

To conclude, the inhibition of dietary fat absorption by PL has been identified as a logically valid and legitimate target at a molecular level to control obesity. Although an endless list of PL inhibitors has been identified and the research is ongoing, none of the natural compounds and dietary phytochemicals has reached the stage of clinical use.

2.9. Wine Polyphenols and Kakhetian Winemaking Method

A great deal of research showed that the class of polyphenols represents one of the essential sources of inhibiting PL activity (Bajes et al., 2020; T. Buchholz and Melzig, 2015; Martinez-Gonzalez et al., 2017). Although individual polyphenols have been shown to possess high anti-lipase activity, some studies indicated that the intake of isolated compounds could have deleterious effects of overconsumption, and it is better to consume them from whole food (Crowe and Francis, 2013; Williamson and Holst, 2008). In addition, the incorporation of polyphenols with anti-obesity potential into food is restricted by low water solubility and poor stability against light, oxygen and heat (Parisi et al. 2014).

In this manner, wine is a complex beverage whose complexity is mainly caused due to the great diversity of polyphenols (Hornedo-Ortega et al. 2021). Grapes and their derivatives are considered one of the richest natural sources of phenolic compounds. Wine polyphenols, together with grape polyphenols, include new phenolic products that are formed during the winemaking process (Gutiérrez-Escobar, Aliaño-González, and Cantos-Villar 2021). Moreover, by drinking wine, individuals consume complex medium, not single polyphenols (Giovinazzo, Carluccio, and Grieco 2019).

Flavonoids and non-flavonoids are the two main types of phenolic chemicals found in grapes and wine. Flavonoids make up the majority of the phenolic chemicals found in red wine and account for more than 85% of the total phenolic content in red wine (Soleas, Diamandis, and Goldberg 1997). Flavanols, flavonols, and anthocyanins are the three major types of flavonoids present in grapes and wines. Flavanols are mostly found in the grape seeds and are presented in the forms of (+)-catechin 13, (–)-epicatechin 14, and proanthocyanidins 20. Flavonols are formed in the skin of the grape, as well as in the leaves and rachis of the grape. Anthocyanins are primarily present in the grape skin and, through condensation with other flavonoids, are responsible for the colour of red wine (Cantos, Espín, and Tomás-Barberán 2002). Hydroxycinnamic and hydroxybenzoic acid derivatives, stilbenes, and so-called hydrolysable tannins are the most common non-flavonoid phenolic chemicals identified in wine (Waterhouse, Sacks, and Jeffery 2016).

The composition of phenolics is highly linked to wine quality properties, such as colour, flavour, and taste, as well as health-promoting properties, including antioxidants and cardioprotective properties, among others (Gutiérrez-Escobar, Aliaño-González, and Cantos-Villar 2021). Moreover, scientific results showed that polymeric polyphenols, which are the main polyphenols in grape and red wine, play a major role in the colour stability, sensory properties and antioxidant activities of wines (Waterhouse, Sacks, and Jeffery 2016). Furthermore, on account of health-promoting chemicals (e.g., phenolics), moderate wine consumption is nowadays recognised as a risk-reducing factor in several human diseases,

including type 2 diabetes (Beulens et al. 2012; Huang, Wang, and Zhang 2017), several types of cancer (Artero et al. 2015) and cardiovascular disease (Arranz et al. 2012; Chiva-Blanch et al. 2013).

Phenolic content in wine can be influenced by many factors such as grape cultivar (Brighenti et al. 2017), soil, climatic conditions and weather (H. M. Shah et al. 2022). The role of the winemaking procedure, conditions of maturation and storage is crucial (Sun et al. 2011).

The country of Georgia is known to use the ancient method of winemaking, which is widely known as the Kakhetian winemaking or Qvevri winemaking method (A Shalashvili et al. 2007). This method differs from Western European techniques, referred to as the Classic winemaking, Industrial approach. Kakhetian winemaking method involves placing crushed grapes and other parts, i.e., clusters (stem, skin, seeds), in a clay vessel called Qvevri that is dug in the ground. Qvevri is then sealed, and the wine is left to mature. Due to this technology, Georgian qvevri wines display numerous chemical properties. For example, during fermentation, phenolic compounds are extracted from pomace, defining the composition and essence of Kakhethian wine (McGovern et al. 2017). Besides the increased level of polyphenols and antioxidants, studies have also indicated enlargement of the unique volatile composition (Martins et al., 2018) and mineral contents caused by the clay contact (Diaz et al., 2013; Joao Cabrita et al., 2018) and the interesting indigenous yeast microbiota (Capece et al. 2013; Vigentini et al. 2016). Bene et al. (2019), showed that not all grape varieties are suitable for Qvevri winemaking in terms of their phenolic substances and health-promoting potential.

Qvevri winemaking method has no analogy in the world, and it has been approved as an intangible cultural heritage convention by UNESCO (UNESCO 2013). Additionally, Georgia is considered the "cradle of wine", as the earliest traces of winemaking have been found here (David Maghradze et al. 2015). The Kakhetian winemaking method can be employed for

both white and red grape varieties. Georgia is home to over 500 varieties of indigenous grapes, many of which are unknown to the rest of the world (Chkhartishvili and Maghradze, 2012). Among them, Saperavi and Rkatsiteli are the most widely planted grape varieties (CENSUS 2014), producing red and white wine, respectively. Both Saperavi and Rkatsiteli are the oldest Georgian varieties, originating in the region of Kakheti (eastern part of Georgia).

Although a significant amount of research has been done about Georgian wines (D. Maghradze et al. 2019; Armaz Shalashvili et al. 2012; Tauchen et al. 2015; Vigentini et al. 2016), to the best of our knowledge, only scarce information about their anti-lipase activity is available. Gulua et al., (2018) studied the chemical constituents, antioxidant and anti-lipase activity of some wines produced in Georgia. However, this study investigated the lipase inhibitory of six different wines, and it did not bring up any relationship between anti-lipase activity and technological methods, nor about the kinetics of inhibition.

In this matter, this research aimed to investigate fourteen wines made from Georgian autogenous grapes (such as Rkatsiteli and Saperavi), obtained through Kakhetian and European winemaking methods and compare their PL inhibitory activity and polyphenol content and find a relationship, if any, between lipase inhibitory activity and winemaking method; elucidate mechanism of inhibition PL by wine.

3. Materials and Methods

3.1 Materials

3.1.1. Wine samples

Samples of commercial wines made from autochthonous and leading grape varieties, such as Rkatsiteli and Saperavi, grown in the region of Kakheti, were chosen for research purposes (CENSUS 2014). In this study, a total of fourteen red and white wines of the Saperavi (n = 9) and Rkatsiteli (n = 5) were assessed. Wines were either provided by local producers or purchased from local wine stores.

The wine samples for the experiment were chosen at random. We did it from the consumer's point of view. As consumers would do, we did it because promising in vivo potent lipase inhibitory activity can be the definitive factor behind consumer decision-making. Investigated samples were dry wines, except for late harvest wines. Most of the red wine samples were Mukuzani (S4-S9), Appellation Controlled Origin (AOC) dry red wine, produced from Saperavi grapes grown in the Mukuzani micro-viticulture area Kakheti region (Sakpatenti 2010). Detailed information about the investigated wines, such as producer, name on the label, vintage, type of wine, alcoholic strength and winemaking method are provided in Table 2. All the wine samples were packed in glass bottles and stored at room temperature until analysed.

Table 2. Wine Characteristics

Wine Code	Name of the bottle	Producer	Vintage	Grape variety	Type	Alcoholic strength %	Wine-making method
S1	Glekhuri - Khasmi Saperavi	Teliani Valley	2017	Saperavi	Dry Red	13	Kakhetian
S2	Matrobela	Matrobela Wines	2018	Saperavi	Dry Red	13.5	European
S3	Icewine -	Guramish	2017	Saperavi	Sweet	12	late-harvest

	Saperavi - Guramishvilis Marani	vili's Marani			Red		(European)
S4	Mukuzani Valley - Mukuzani	Mukuzani Valley	2016	Saperavi	Dry Red	12.5	European
S5	Mukuzani Valley - Mukuzani	Mukuzani Valley	2019	Saperavi	Dry Red	12.5	European
S6	Rtvelisi - Mukuzani	Rtvelisi	2018	Saperavi	Dry Red	13	European
S7	Zurab Tsereteli - Mukuzani	Tsereteli Wine and Spirits	2015	Saperavi	Dry Red	13	European
S8	Zhamurashvili's wine - Mukuzani	Zhamurashvili's wine	2018	Saperavi	Dry Red	13	Kakhetian
S9	Nekresi Estate - Mukuzani	Nekresi winery	2016	Saperavi	Dry Red	13	Kakhetian
RK 1	Icewine Satrapezo	Telavi Wine Cellar	2013	Rkatsiteli	Sweet White	10.5	Late harvest (European)
RK 2	Rkatsiteli Vine Ponto	The Spirit of Georgia	2016	Rkatsiteli	Dry White	12.5	Kakhetian
RK 3	Mr Rkatsiteli from Gurjaani	Mr Wine	2018	Rkatsiteli	Dry White	13	Kakhetian
RK 4	Rkatsiteli – Vaziani	Vaziani company	2016	Rkatsiteli	Dry White	12.5	European
RK 5	Rkatsiteli	Kindzmaruli's Marani	2018	Rkatsiteli	Dry White	13	European

3.1.2. Chemicals

Detergent Tween 80, Olive Oil, Sodium Hydroxide, Folin-Ciocalteu reagent, ethyl acetate, Gallic acid, and methanol were purchased from Sigma – Aldrich (Steinheim, Germany), TPTZ-2,4,6-Tris (2-pyridyl)-s-triazine from Swiss Sigma – Aldrich, Lipase concentrate – H.P. was purchased from Integrative Therapeutics, LLC (USA). Orlistat® (trade name Xenical) by Roche (Italy) was purchased at the local pharmacy. All other chemical reagents were available at the local market and were of high purity.

3.1.3. Laboratory Utensils and Equipment

Following laboratory utensils were used in this research: Burettes, evaporating (porcelain) dishes, pipettes, graduated cylinders, volumetric flasks (25, 50, 100, 500, 1000 ml capacity), Erlenmeyer flasks, beakers, glass tubes (10ml capacity), graduated cylinders (10, 50, 100, 500 ml capacity), test tube racks, tong, glass desiccator, thermometer, tape, stopwatch, magnetic stirrer.

Research instruments and equipment include:

- Automatic titrator - ZDJ-4A, Automatic Potential Titrator, NASA Scientific Instrument Co., Ltd, CHN (pH Accuracy ±0.01pH);
- Digital Lab Scale - Balance - capable of weighing to an accuracy of ±0.001 g. Shanghai Yoke Instrument Co. Ltd, CHN Water bath - LW-4, 800W WSL BYTOM Ltd, PL;
- Digital reading micro and macro pipettes, Acura manual 825 and 835, respectively. Socorex Isba SA Ltd, CH.
- Drying cabinet - BOV-TC, Biobase Ltd., CHN;
- Rotary Vacuum Evaporator – RE 2000B, Henan Lanphan Technology Co. Ltd. CHN;
- Spectrophotometer – UV 1609, AandE Lab Co. Ltd, UK;

3.2. Methods

3.2.1. Winemaking methods

Wines included in this study were either made based on the common "European methods," i.e., Classic technology or the Kakhetian method, i.e., Qvevri technology. Wines made with different technology were chosen in an attempt to find a correlation, if any, between lipase inhibitory activity and winemaking methods.

3.2.2 Titratable acidity

Titratable acidity (TA) of wine samples was determined by titration with 0.1 N sodium hydroxide to a defined endpoint using an automatic titrator. Tartaric acid is considered as the most prominent acid in wine. Thus, TA results were expressed in grams of tartaric acid per litre. The endpoint of pH 8.2 was chosen for the same reason. Therefore, the titration determines the amount of organic acids that can be neutralised by a sodium hydroxide solution at pH 8.2 (Zoecklein et al. 1990) and can be calculated by the following equation:

$$Titratable\ acidity\ \left(\frac{g}{1000mL}\ tartaric\ acid\right) = \frac{\mathrm{N}*\mathrm{V1}*0.075*1000}{V2} \quad /1/$$

Where N equals to the normality of titrant (mEq/mL), V_1 is the volume of titrant (mL), V_2 - the volume of the sample, 0.075 is mEq weight of tartaric acid, 1000 conversion factor of milliequivalents to equivalent mass.

Before titration, the carbon dioxide was removed since it can cause errors of over 1g/L. Thus, wine samples were subjected to a strong vacuum. To measure the titratable acidity, 10 mL of degassed sample was pipetted into a 150 ml glass beaker, and distilled water was added to make sure that the total volume was sufficient to cover the pH probe.

3.2.3. Total dry extract

For measurement of non-volatile dry matter, a 50 mL sample of wines was aliquoted into a porcelain dish. The dish was then placed onto a boiling water bath until the evaporation of water, alcohol, and other volatile compounds had occurred. The residual moisture was then evaporated from the samples by oven drying at 105^0C for 16h. Finally, the total dry extract was determined gravimetrically as the residue remaining after drying (Bradley 2010).

3.2.4 Determination of total phenolic content

The total phenolic content (TPC) was determined spectrophotometrically, by using the Folin-Ciocalteu method (Singleton, Orthofer, and Lamuela-Raventós 1999). This is a classical determination method for TPC, which like every other Colorimetric assay, is low-cost, rapid, and easy to perform (Zhu and Gao, 2019). Furthermore, this method is referenced by the International Organisation of Vine and Wine (OIV) and used in the European Union (EU) as the official method of analysis. All phenolic compounds in wine are oxidised by the Folin-Ciocalteu reagent, and blue colour is formed, which can be quantified by visible-light spectrophotometry.

Total phenolic contents in the samples were expressed as $\mu g \cdot mL^{-1}$Gallic acid equivalent (GAE). Diluted Gallic acid (10-50 $\mu g \cdot mL^{-1}$) was used as a working standard solution to plot a relationship between standard optical densities and Gallic acid concentrations. Gallic acid as standard was chosen due to its stability and purity. It is also less expensive than other options. Furthermore, it has been proven that the response to Gallic acid is equivalent to most other phenolics in wine on a mass basis (Hakim 2019).

To determine the TPC, 1 mL of diluted wine samples were added into separate glass tubes. Next, 5 mL of Folin-Ciocalteu phenol reagent (1:10 v/v distilled water) was pipetted into

each tube. The diluted Folin-Ciocalteu phenol reagent was prepared on the day of use. The reagent itself was stored in the refrigerator at 4^0C and allowed to reach room temperature before use.

The mixture was left for 8 minutes to allow the reaction before the addition of 4 mL of Sodium Carbonate solution (7.5% (w/v)). The tubes were stoppered, and the solutions mixed well. Samples were allowed to stand for another 60 minutes at room temperature for colour development. Then their optical densities against the water were read at 765 nm, with a 10 mm path length cell.

3.2.5 Ferric reducing antioxidant power (FRAP) assay for total antioxidant activity

Various methods have been developed to assess the antioxidant activity (AOA) of foods (Zhong and Shahidi, 2015). In this research, ferric reducing antioxidant power (FRAP) assay has been applied to evaluate the total AOA of samples.

The FRAP assay measures the antioxidant power based on the ability of low pH to reduce ferric-tripyridyltriazine (Fe^{3+} -TPTZ) to ferrous-tripyridyltriazine (Fe^{2+} -TPTZ) complex and form an intense blue colour. The change in absorbance is measured at 593 nm (Benzie and Strain, 1996). Originally it was used to assess the reducing power in plasma, but later its usage was applied in the measurement of antioxidant activity in other biological fluids, foods, and plant extracts. The FRAP method is feasible, rapid, cost-effective, and can be performed without specialised equipment (Zhong and Shahidi, 2015). Furthermore, this assay has been proven to measure the antioxidant capacity of foods which is closely related to their polyphenol content (Galili and Hovav, 2014).

FRAP assay was carried out according to Benzie and Strain (1996), with slight modifications. The working FRAP reagent was prepared freshly by mixing 300 mM acetate buffer (pH 3.6)

with 10 mM 2,4,6- tripyridyl-s-triazine (TPTZ) solution (dissolved in 40 mM HCl) and 20 mM Ferric Chloride solution in the ratio 10:1:1. The FRAP reagent was incubated at 37^0C for 15 minutes. The absorbance of 3 mL of working reagent was read at 593 nm. Then it was mixed with 100 microliters of the diluted sample. The absorbance change was monitored, and the optical densities were recorded after 4 min. 1mM ascorbic acid was used as a positive control. FRAP values of samples were compared to that of ascorbic acid and expressed as mg ascorbic acid equivalents (AAE) per litre of wine.

3.2.6 Determination of Lipase Activity

Numerous methods are established to determine lipase activity, i.e., volumetric, spectrometric, conductimetric, and chromatographic techniques. In addition, biosensor-based methods, radioactive assays and immunoassays are also applied to evaluate lipase activity. The above-mentioned methods differ regarding their fundamental principle, chosen substrates, sensitivity, and applicability (Stoytcheva et al. 2012).

The titrimetric assay was chosen to determine lipase activity because of its simplicity and accuracy. Even though the titrimetric assay is time-consuming (two determinations per hour) and errors can occur due to incomplete titration, this method remains in use (Stoytcheva et al. 2012). Furthermore, according to Gupta et al., (2003), the titrimetric assay is the most reliable and widely used procedure. This method is based on the titrimetric determination of the free fatty acids released from triacylglycerols by lipase catalysed hydrolysis. The procedure involves sample incubation and end-point titration of the liberated acids. Results are dependent on lipase activity (Stoytcheva et al. 2012).

The method reported by Sigma Aldrich (SigmaAldrich 1997) was applied to determine lipase activity. The initial reaction mixture contained 2.5 mL of deionised water, 1 mL 200 mM Tris HCl buffer (pH 7.2), 3 mL of olive oil, and 0.5 mL of detergent (Tween 80). Experimental procedure was modified by vigorously mixing the solution on a magnetic stirrer for 15 min.

The modification was applied to obtain a good emulsion. Subsequently, 110 mg of the lipase concentrate was added to the emulsified mixture, which was then incubated at 37 ^{0}C for exactly 30 min. At the end of the incubation, 3 mL of 95% ethanol was added. The total volume was then brought to 135 mL by adding distilled water and the final reaction mixture was titrated with 50 mM NaOH until the value of pH 9 at automatic was achieved. A pH of 9 was chosen as the end-point because the complete ionisation of free fatty acids occurs at this value (Cherry and Crandall, 1932; Hoppe and Theimer, 1996). Blank titration was performed as above without incubation and lipase in the test sample. The obtained results were used to calculate Lipase activity.

One unit of lipase activity (LA) is defined as the amount of enzyme that hydrolyses 1.0 micro equivalent of fatty acid from a triglyceride in one hour at pH 7.2 at 37 °C. The following equation was used to calculate LA:

$$\text{Lipase Units/mg enzyme} = \frac{(A - B)\,(1000)\,(2)\,(DF)}{(1)} \qquad /2/$$

where A = volume of 50 mM NaOH consumed by the test sample in mL;

B = volume of 50 mM NaOH consumed by the blank sample in mL;

1000 = conversion factor from milli equivalents to micro equivalents;

2 = time conversion factor from 30 min to 1 hour;

DF = dilution factor;

1 = amount (in mg) of the used enzyme (PL).

3.2.7 Determination of Lipase inhibitory activity of wines

To exclude the impact of alcohol on the lipase, de-alcoholised red and white wine samples were used in the lipase inhibition assay. Alcohol was removed on the rotational evaporator at 40 ^{o}C (Shalashvili et al. 2012). To measure the percentage of lipase inhibition, 1 mL of double concentrated wine sample was added to the initial mixture; the following procedures were identical to those described previously (subchapter 3.2.6). The percentage of inhibition was

calculated in the presence and absence of inhibitors. Orlistat was used as a standard inhibitor. In this case, lipase activity was measured in the presence of Orlistat (10mg), and the percentage of inhibition was calculated per 1 mg of Orlistat.

3.2.8 Liquid-liquid Extraction of Wine

The liquid-liquid extraction method was used to fractionate phenolic compounds of chosen wine (Roussis, Lambropoulos, and Soulti 2005). After extraction, aqueous and organic phases were obtained. According to Roussis et al., (2005), the aqueous phase mainly contains anthocyanins and polymerised phenolic compounds. The ethyl acetate fraction contains plenty of flavanols, flavonols, and phenolic acid. Wine phenolic extracts were used to study the kinetic behaviour of each fraction individually and determine their combined effect on the activity of PL.

To obtain phenolic extracts, firstly, alcohol was removed from wine by the rotary evaporator at 40 ^{o}C (Salagoïty-Auguste and Bertrand, 1984). Ethyl acetate was used as solvent, because, according to Pintać et al. (2018) ethyl acetate is the most efficient solvent for a wide range of phenolic classes. The pH of dealcoholised wine was then brought up to 2 and extracted by ethyl acetate. Ethyl acetate was then evaporated under a vacuum (30 ^{0}C) and the organic phase was redissolved in distilled water. Organic phase was extracted again with ethyl acetate and subsequently followed by evaporation of ethyl acetate. With this process aqueous and organic (ethyl acetate) phases (fractions) were obtained (Shalashvili et al. 2012). The ethyl acetate fraction was dissolved in ethanol. Obtained phenolic extracts were subjected to further analysis. To exclude the impact of ethanol on the lipase in the lipase inhibition assay, ethanol was removed from the ethyl-acetate fraction.

3.2.9 Evaluation of Pancreatic Lipase Inhibition Kinetics

The kinetic analysis of PL activity inhibited by wine and its fractions was determined by using the graphical method via double reciprocal (Lineweaver-Burk) plots (Bhagavan 2002). The plots were set up at different olive oil concentrations varying from 0.5 to 3mL for the PL reaction with and without wine and wine fractions. The concentration of inhibitors and PL were kept constant. The mode of inhibition (MOI) was determined by looking at the pattern of interception and crossing of linear lines for the reciprocal data of PL activity with and without inhibitors vs olive oil concentration.

Enzyme kinetics can be governed by the following Michaelis-Menten equation (eq. 3), where K_m represents the Michaelis-Menten constant and indicates the substrate-binding affinity. V_{max} stands for the maximum reaction rate, which is also called enzyme activity. These kinetic parameters were calculated using the Lineweaver-Burk equation (eq. 4), which is the reciprocal of Michaelis-Menten Equation. The Lineweaver-Burk equation can be compared with the equation for a straight line: y = ax + b, in which "b" (the y-intercept) is equal to the $1/V_{max}$ and "a" (the slope) is equal to the value of K_m/V_{max} (Roskoski 2015). The value of K_m/V_{max} is also known as specificity time (Cornish-Bowden 2012). K_m and V_{max} in Eq. 3 refer to the K_m and V_{max} value of inhibition, which in the inhibition study can also be denoted as apparent (app) values - $K_{m,\ app}$, and $V_{max,\ app.}$ The inhibition constant (K_i) was calculated according to the MOI.

$$V = \frac{V_{max}[s]}{K_M + [s]} \qquad /3/$$

$$\frac{1}{V} = \frac{K_m}{V_{max}} \frac{1}{[s]} + \frac{1}{V_{max}} \qquad /4/$$

where [V] stands for the reaction velocity, [V_{max}] – the maximum reaction velocity, [K_m] – Michaelis-Menten constant [S] substrate concentration.

3.3 Statistical analysis

Statistical analyses were performed with Microsoft Excel (Microsoft 365 MSO, Version 2112, statistical functions, Microsoft Corp., Redmond, WA, USA). Presented data are the mean of a minimum of three replicates ± standard deviation (SD). Data were subjected to the one-way ANOVA and Tukey's HSD tests.

One-way analysis of variance (ANOVA) was done to analyse the significance of the variation of means between the control and the experimental samples.

The Tukey's honestly significant difference (Tukey's HSD) test was used to differentiate between the mean values and find the significant difference, if any, among them at $P< 0.05$ and $P < 0.001$.

The data analyses were done, also using XLSTAT (free trial version 2022, Addinsoft, Inc., Brooklyn, NY, USA).

The LINEST function was used to calculate the kinetic data. This function fits known data points to the straight line by the least square method and returns the statistics of that.

4. Results and Discussions

4.1 Chemical Constituents

The wines included in this study were either made based on the common "European methods" or by the Kakhetian method. The specificities of winemaking technologies along with climatic conditions and grape varieties play a major role in distinct diversities among Georgian wines. These factors influence chemical composition of wines, i.e. polyphenol content, which in turn may have an influence on the anti-lipase activity of wines (Glonti 2010). Therefore, before assessment of lipase inhibitory activity of Georgian wines, different chemical and biochemical parameters were evaluated.

The chemical characteristics of wines studied herein are shown in Table 6 (see appendix A). Variability in the constituents of the different wines were observed by compositional analysis.

4.1.1 Titratable Acidity

The results of three measurements for each wine, with error bars are presented on the figure 5 below. The total titratable acidity varied between 4.791 and 7.986 $g \cdot L^{-1}$ tartaric acid equivalent for white wines and between 5.25 - 8.209 gram tartaric acid equivalent per litre for red wines. The established titratable acidity for Mukuzani wines provided by the legislation of Georgia has to be greater than 5 $g \cdot L^{-1}$ of tartaric acid (Georgian Government 2019). All of the investigated Mukuzani wine samples met this requirement. According to the same resolution, titratable acidity of table wines should equal or exceed 4 $g \cdot L^{-1}$ of tartaric acid. All the wine samples obeyed this rule.

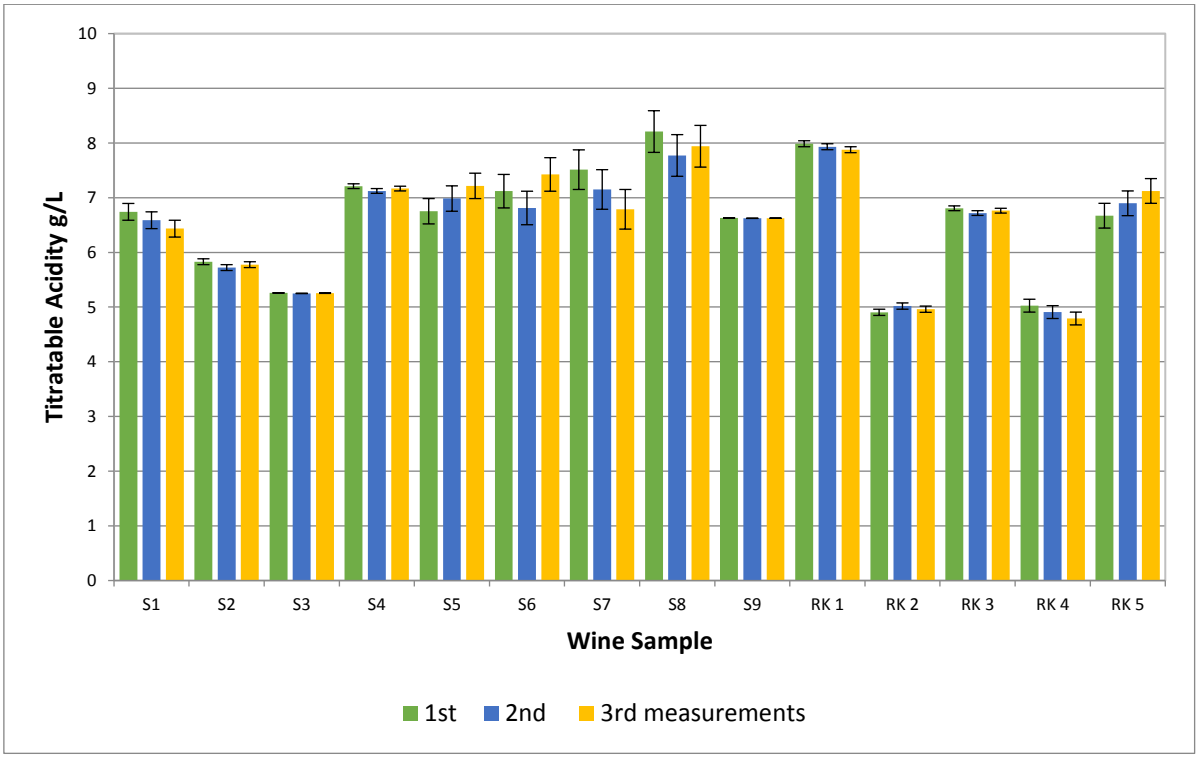

Figure 5. Titratable acidity of different wine samples, with error bars.
S1- Glekhuri, Khasmi Saperavi; S2 - Matrobela, Saperavi; S3 - Icewine, Guramishvilis Marani, Saperavi; S4 -Mukuzani Valley, Mukuzani (2016); S5 - Mukuzani Valley, Mukuzani (2019); S6 - Rtvelisi, Mukuzani ; S7 - Zurab Tsereteli, Mukuzani; S8- Zhamurashvili's wine, Mukuzani; S9 - Nekresi Estate, Mukuzani; RK1 - Icewine Satrapezo, RK2 – Rkatsiteli, Vine Ponto; RK3 - Mr Rkatsiteli from Gurjaani; RK4 - Vaziani, Rkatsiteli; RK5 - Rkatsiteli;

4.1.2 Dry Extract Content

The highest total dry extract was presented in late-harvest wines, 100.54 ± 0.06 $g \cdot L^{-1}$ in Saperavi and 97.24 ± 0.04 06 $g \cdot L^{-1}$ in Rkatsiteli wines. Compared to the other samples, these values were significantly higher. Among the other samples, white wines made through the common European method (Rk4 and Rk5) contained the lowest amount of total dry extract, 13.46 ± 0.15 and 16.62 ± 0.18 $g \cdot L^{-1}$, respectively. Non-volatile matter in the white wine sample Rk3 was found to be 20.36 ± 0.02 $g \cdot L^{-1}$. Total dry extract content in the rest of the samples varied between 24.32 and 30.69 $g \cdot L^{-1}$. The high content of dry extract in late harvest wines can be explained by winemaking process, since they belonged to ice-wine. Ice-wine is a type of dessert wine produced from fully ripened and naturally dehydrated grapes that have been frozen while still on the vine. Usually, grapes are left onto vine until the

temperature drops below -9⁰C. This pre-harvest dehydration concentrates the soluble solids in grape berries. As a result, wine is rich in sugars, phenolic compounds and flavour (Moreno et al., 2008).

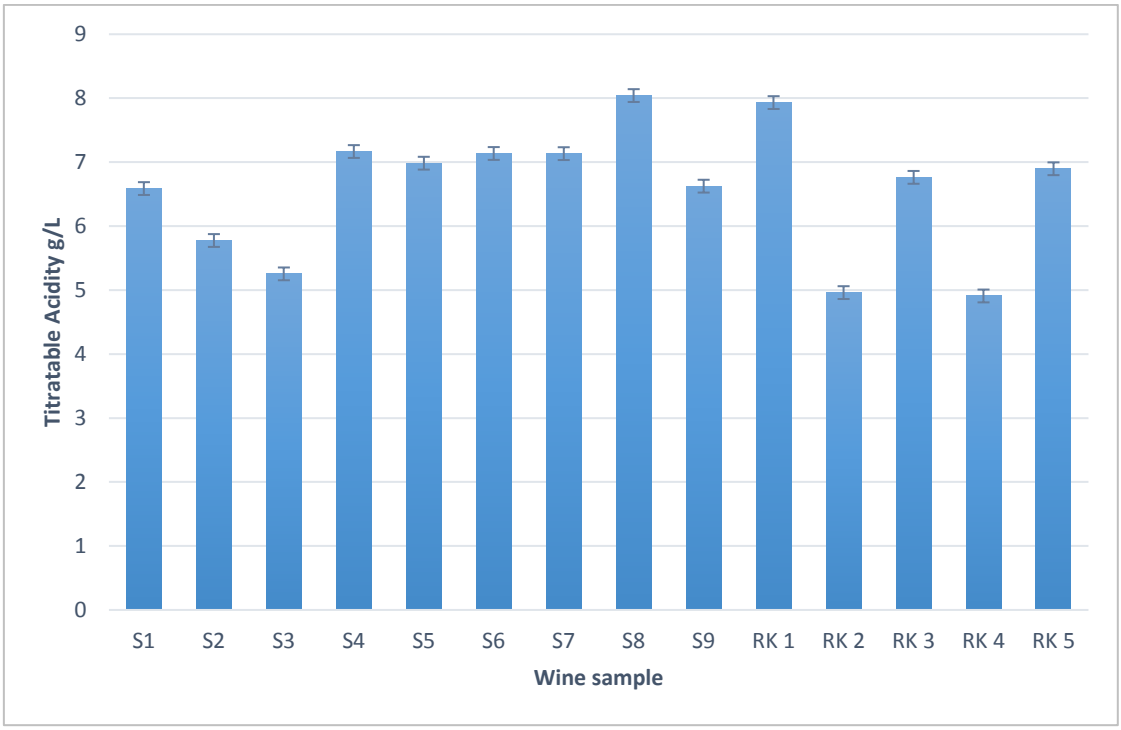

Figure 6. Dry Extract content g/L in different wine samples. Means of three replicates, with error bars. S1- Glekhuri, Khasmi Saperavi; S2 - Matrobela, Saperavi; S3 - Icewine, Guramishvilis Marani, Saperavi; S4 -Mukuzani Valley, Mukuzani (2016); S5 - Mukuzani Valley, Mukuzani (2019); S6 - Rtvelisi, Mukuzani ; S7 - Zurab Tsereteli, Mukuzani; S8- Zhamurashvili's wine, Mukuzani; S9 - Nekresi Estate, Mukuzani; RK1 - Icewine Satrapezo, RK2 – Rkatsiteli, Vine Ponto; RK3 - Mr Rkatsiteli from Gurjaani; RK4 - Vaziani, Rkatsiteli; RK5 - Rkatsiteli;

4.2 Total Phenolic Content

Phenolic compounds are one of the most important quality parameters of wines. A critical role of these compounds is determined by their contribution to the organoleptic characteristics, such as colour, astringency, and bitterness (L. Li and Sun, 2019). Additionally, they are linked to the health-promoting properties of wine (Lucarini et al. 2021).

Before determination total phenolic content within the samples, calibration curve was plotted. Diluted Gallic acid (10-50 μg·mL^{-1}) was used as a standard compound to plot a relationship between optical density and Gallic acid concentration. The increase in absorbance is caused by the intensity of colour developed and is directly proportional to concentration of standard for total phenolics (Bond et al. 2003). The obtained chart equation was as follows y = 0.0123x + 0.0236, where "y" is the optical density at 765 nm and "x" is Gallic acid concentration μg·mL^{-1}. Pearson's correlation coefficient (R2) for this equation was equal to 0.9918.

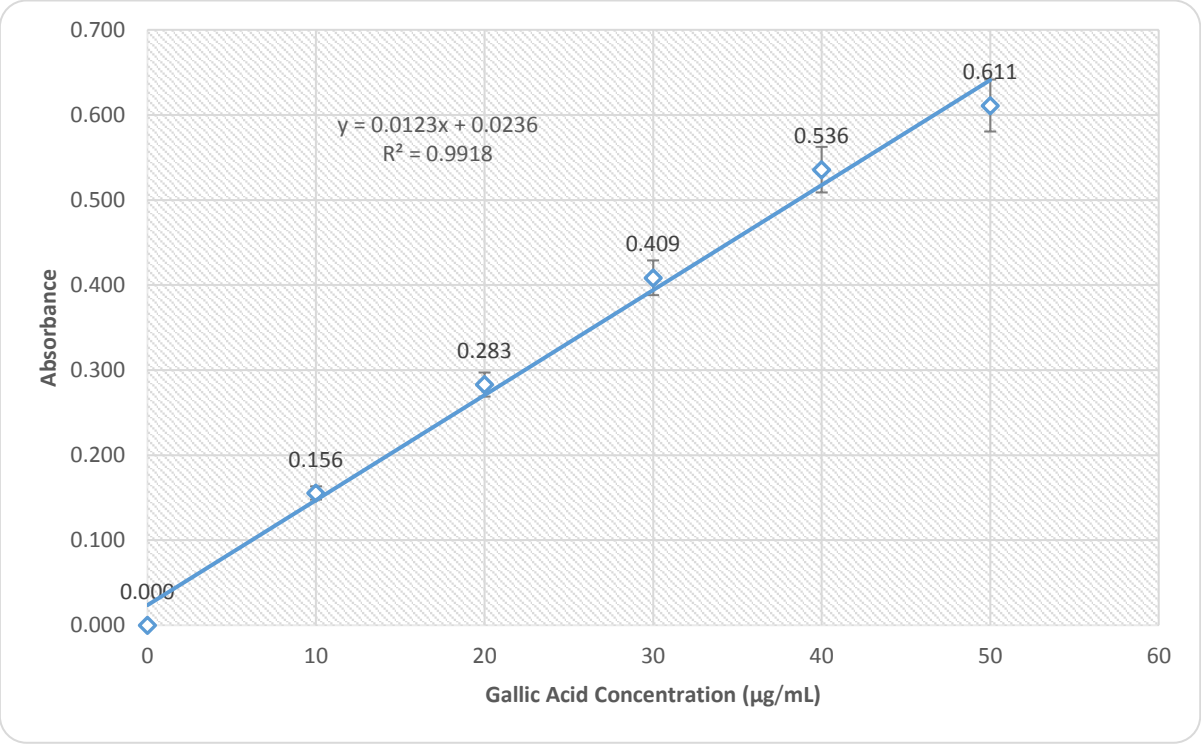

Figure 7. Calibration curve of standard Gallic acid for determination of total phenolic content

The obtained standard curve was used to determine the total phenolic content in wine samples. It is worth mentioning that the optical densities of samples were within calibration range, as deviation from linearity can occur at higher concentrations. In order to fit optical densities within this range, measurements were made at different dilutions of wine samples. Obtained results are shown in table 4 and confirm a variation in TPC among tested wine samples.

In general, the amount of phenolic contents of wines is determined by the grape variety, environmental factors and wine making techniques (Giovinazzo, Carluccio, and Grieco 2019). The highest total phenolic content was observed in S5 (Mukuzani Valley, 2019) and S2 (Matrobela wines) samples, i.e., 3572.358 ± 187.521 and 3482.927 ± 136.204 mg GAE L^{-1}, respectively. The TPC among the rest of Saperavi grape wines varied between the range of 2415 and 2930 mg GAE L^{-1}. Compared to the other red wine samples, Zurab Tsereteli's Mukuzani contained a significant amount of TPC, 2415.176 ± 19.163 mg GAE L^{-1}, which could be caused by the winemaking method or vintage, or both. The TPC of this sample was significant to the white wine sample RK 2 (Rkatsiteli Vine Ponto), fermented by the Kakhetian method. The presence of high concentrations of TPC in white wine produced by the Kakhetian method was expected due to skin-contact fermentation. This is a good example of how the production method can increase the TPC in wine. Usually, consumers consider that white wines do not have polyphenols present in such large quantities as red wine; however, due to the specificities of Kakhetian winemaking method, a white wine made with this method can have comparable TPC to some red wines made by classical, European method.

Phenolic content in various white wines was significantly different. The highest total phenolic content was found in the Rkatsiteli sample brand Vine Ponto 2515.447 ± 137.972 mg/L GAE, this value was comparable to some of red wines (S6, S1, S7, S3). The TPC of the samples from brands Vaziani and Qindzmarauli Marani were as follows 489.577 ± 36.112 and 190.243 ±11.498 mg/L GAE. In general, (as seen from the table) white wines made through the Kakhetian method (RK2 and RK 3) possessed significantly higher TPC in comparison to the rest of the white samples, prepared by the common European method (RK1, RK4, RK5). During fermentation in qvevri vessels, phenolic compounds are extracted in large quantities from the stem, peel and grapes, which explains the reason why white wines of Kakhetian

type showed the highest phenolic content than those of European type. Similar results were reported by (Bene et al. 2019; Gulua et al. 2018; A Shalashvili et al. 2010).

Summary of all pairwise comparisons for Wine samples are shown in the table 3. Additionally, graphical representation is shown in Fig.8.

Table 3. Total phenolic content expressed in terms of Gallic acid equivalent (mg/L) in different wine samples

Wine Code	Total Polyphenol Content $\mu g \cdot mL^{-1}$ GAE
S5	3572.358a
S2	3482.927a
S9	2965.312b
S4	2930.081b
S8	2813.550b
S6	2810.840bc
S1	2734.959bc
S7	2415.176bc
RK2	2035.772cd
S3	1828.455de
RK3	1572.358e
RK4	499.187f
RK5	190.244g
RK1	149.593g

Parameters followed by the same letter in each variety were not significantly different at $p < 0.0001$. S1- Glekhuri, Khasmi Saperavi; S2 - Matrobela, Saperavi; S3 - Icewine, Guramishvilis Marani, Saperavi; S4 -Mukuzani Valley, Mukuzani (2016); S5 - Mukuzani Valley, Mukuzani (2019); S6 - Rtvelisi, Mukuzani ; S7 - Zurab Tsereteli, Mukuzani; S8- Zhamurashvili's wine, Mukuzani; S9 - Nekresi Estate, Mukuzani; RK1 - Icewine Satrapezo, RK2 – Rkatsiteli, Vine Ponto; RK3 - Mr Rkatsiteli from Gurjaani; RK4 - Vaziani, Rkatsiteli; RK5 - Rkatsiteli;

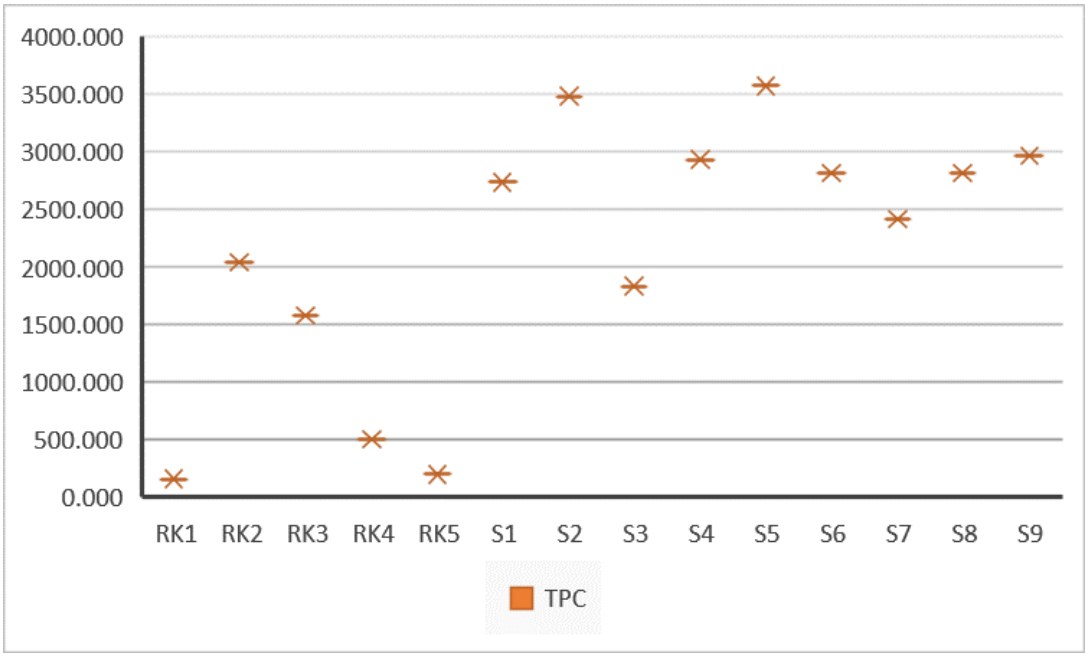

Figure 8. The means of total phenolic content expressed in terms of Gallic acid equivalent (mg/L) in different wine samples

4.3 Ferric Reducing Antioxidant Power of Wines

In the FRAP assay white wines made by classic European technology showed significantly lower antioxidant power than the red wines. However, wines fermented through the Kakhetian method exhibited higher antioxidant activity and were comparable to some red wines made by the common "European method".

The wine S5 exhibited the highest AOA, 4729.199 ± 88.162 mg AAE L^{-1}. Wine S2 showed the second-highest antioxidant activity, 4160.465 ± 126.339 mg AAE L^{-1}. No statistically significant difference was detected between the samples S9, S1, S4, S8 and S6. The observed AOAs were 3494.381 ± 94.199, 3397.031 ± 194.837. 3371.412 ± 240.218; 3299.68 ± 88.152; 3145.968 ± 113.186 mg AAE L^{-1}, respectively. The sample S7 showed no statistically significant difference compared to the sample S6, which was 3012.751 ± 119.724 mg AAE L^{-1}. The antioxidant activity of white wine sample, RK2 (2413.275 ± 53.247 mg AAE L^{-1}), was higher than red wine sample S3 (1921.397 ± 146.631 mg AAE L^{-1}). The difference in AOA between these samples was statistically significant. However, the AOA of this late-harvested

Saperavi wine was statistically similar to that of RK 3 (1788.181 ± 84.658 mg AAE L^{-1}). As stated earlier, wines fermented by the European method, RK1, RK5 and RK4 showed the lowest antioxidant activity; results were statistically similar and varying between 179 and 211 mg AAE L^{-1}. Comparing the AOA of the samples, one can conclude that winemaking technology plays an important role in the antioxidant activity of white wines.

Similar results were observed by Tauchen et al. (2015) when comparing in vitro antioxidant activities of Georgian, central and west European wines. According to this study, wine made with Kakhetian method exhibited antioxidant activity comparable to some red wines and showed higher antioxidant efficacy than the rest of the white wine samples.

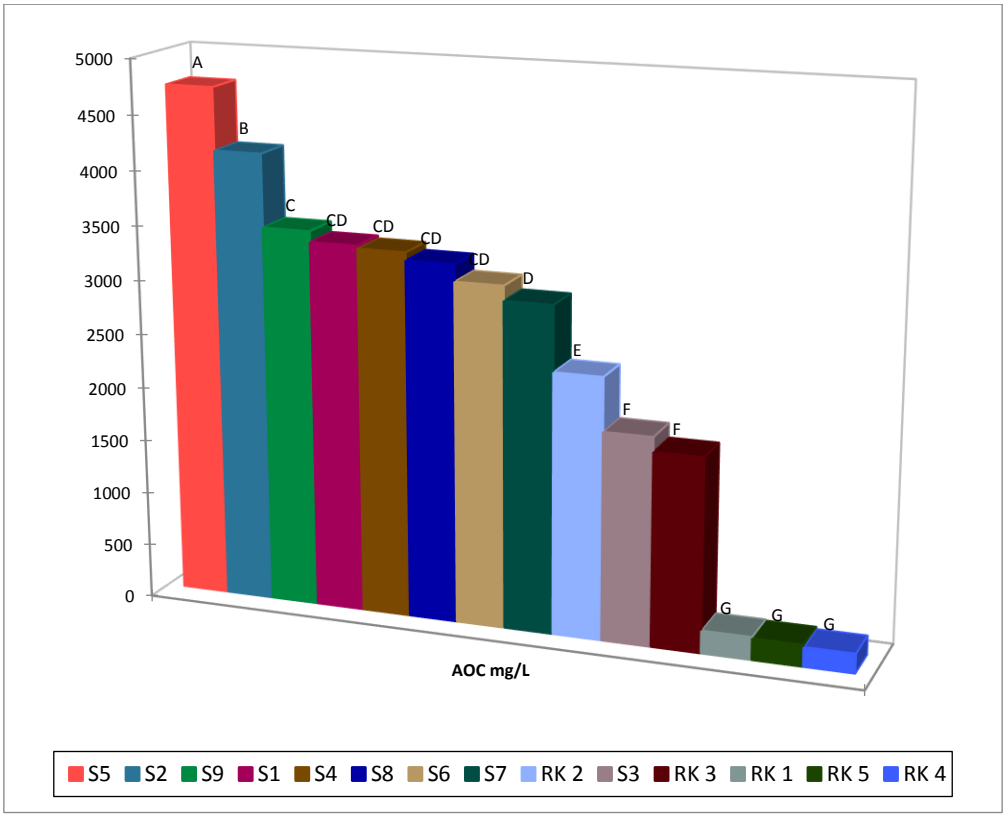

Figure 9. Antioxidant activity of different wine samples expressed in terms of ascorbic acid equivalent (mg/L). Different letters on bar chart indicates significance difference at the p < 0.0001 probability level. S1- Glekhuri, Khasmi Saperavi; S2 - Matrobela, Saperavi; S3 - Icewine, Guramishvilis Marani, Saperavi; S4 -Mukuzani Valley, Mukuzani (2016); S5 - Mukuzani Valley, Mukuzani (2019); S6 - Rtvelisi, Mukuzani ; S7 - Zurab Tsereteli, Mukuzani; S8- Zhamurashvili's wine, Mukuzani; S9 - Nekresi Estate, Mukuzani; RK1 - Icewine Satrapezo, RK2 – Rkatsiteli, Vine Ponto; RK3 - Mr Rkatsiteli from Gurjaani; RK4 - Vaziani, Rkatsiteli; RK5 - Rkatsiteli;

4.4 Correlation between TPC and AOA

The antioxidant activity of wine is mainly dependent on its phenolic content and composition. A significantly positive correlation was reported between the antioxidant activity of Spanish wines and the total phenols or the total anthocyanins (Moreno-Montoro et al. 2015).

The phenolic content itself is determined by the phenolic composition of grapes used (Singleton and Esau 1969), the vinification process (Linskens and Jackson 1988; Sun et al. 2011) and the maturation process (Gómez-Plaza et al. 2002; Niculescu, Paun, and Ionete 2018).

To demonstrate the direction, and form of the relationship between TPC and AOA scatter plot was obtained. As it can be seen from the Fig. 10, linear relationship was displayed between two variables. Additionally, positive correlation can be observed from the scatter plot since the movements of variables are positively linked, and the data points make a straight line going from near the origin out to high y-values. A significantly positive correlation between TPC and AOA was also reported by other researchers (Gulua et al. 2018; Han et al. 2017; Paixao et al. 2007).

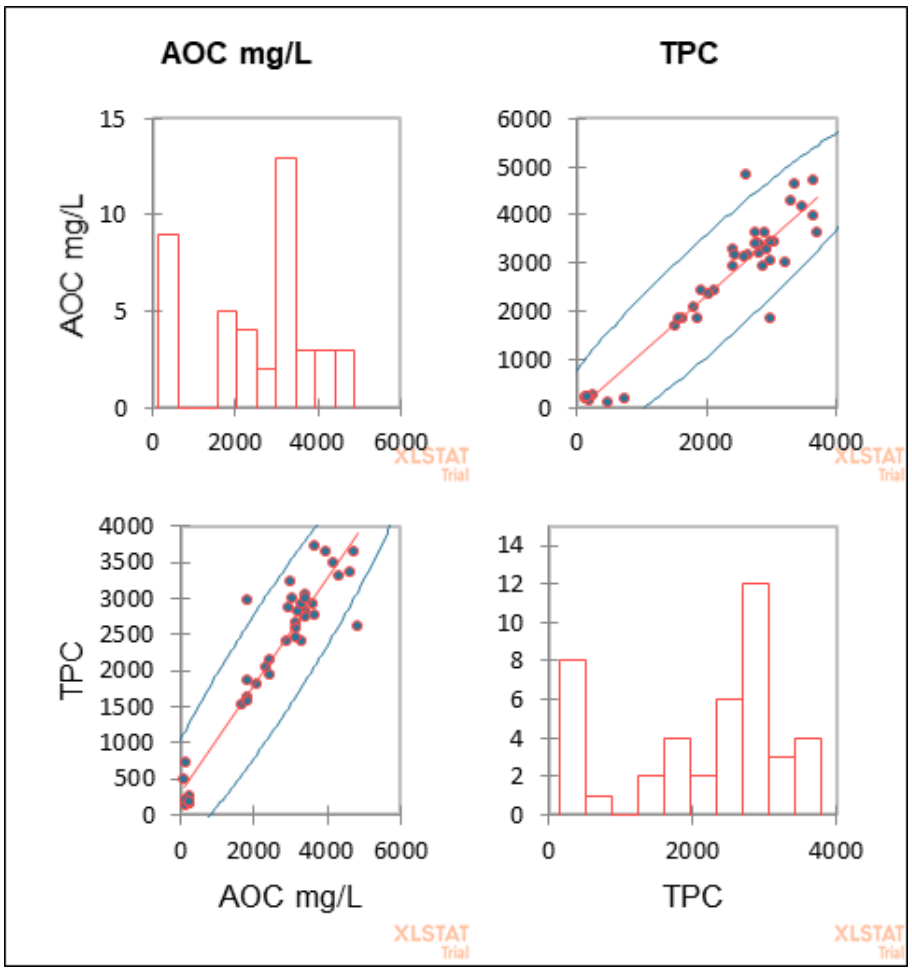

Figure 10. Schematic representation of correlation between the total phenolic content and antioxidant activity of different wines

Additionally, to measure the strength of the relationship, correlation coefficient was obtained from Fig. 10. The R2 was equal to 0.9731, indicating a strong correlation between the total polyphenol content and the antioxidant activity of wines.

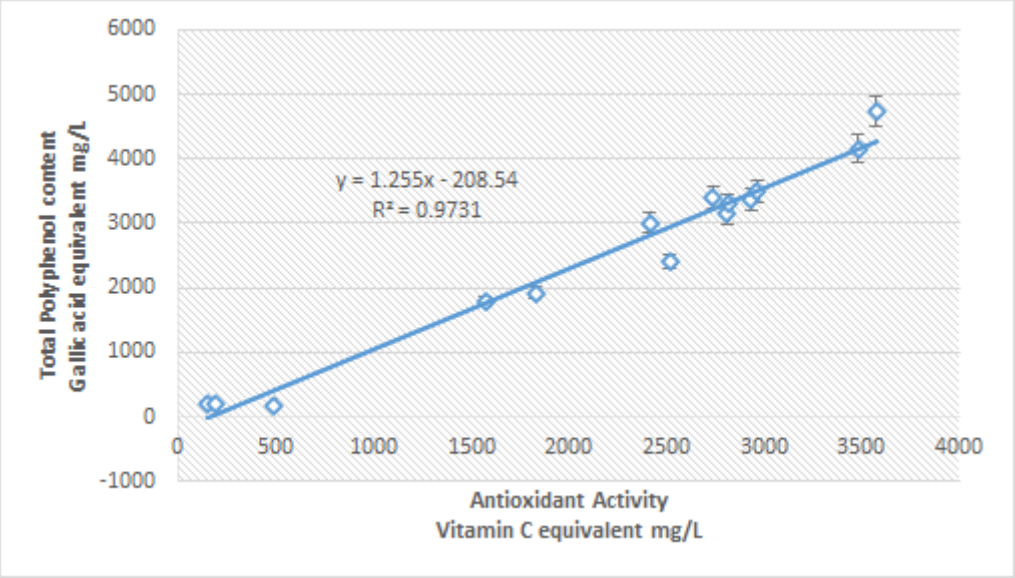

Figure 11. Correlation between the total phenolic content and antioxidant activity

4.5 Lipase Activity

In order to determine in vitro lipase activity with the presence of inhibitors, 1 mL of double concentrated wine samples were added into the PL reaction mixture. In other experiment 10 mg of Orlistat was incorporated to the PL reaction mixture. The experimental data and summary of all pairwise comparisons of lipase inhibition are shown in Fig. 12.

The mean of lipase activity of blank sample was equal to 2018.77 ± 90.45 lipase units/mg enzyme. The lower lipase activity was observed in the rest of the samples due to the presence of PL inhibitors. Overall, wines made with European winemaking method were less capable to inhibit lipase activity. The highest number of lipase units per mg enzyme were observed in Rkatsiteli wine samples (RK4 and RK5) that was as follows 1820.5 ± 74.31; 1416.77±57.84; meaning that compared to the other wine samples they were less capable to slow down the enzyme reaction. These wines were produced by the common "European method" and also possessed relatively small TPC and AOA compared to the other samples. These two samples were followed by wine samples RK1 and S2, also prepared according to the classic European technology. No significance difference was observed between the lipase activity of these

samples and the amount of enzyme that hydrolysed 1.0 micro equivalent of fatty acid from a triglyceride in one hour was equal to 1187.48 ± 48.48 and 1125.42 ± 45.95, respectively. No statistically significance difference was observed between S3, RK2 and S4 samples and the units of lipase activity were as follows: 1009.21 ± 34.47, 939.01 ± 33.43 and 923.62 ± 36.71. It is worth mentioning that white wine (RK2) made with Kakhetian winemaking method was able to slow down lipase activity as much as the red wines, processed by European method. Lipase activity of the rest of the red wine samples did not differ, since they were able to weaken lipase activity equally. These wines were made from Saperavi grape variety, and except for S1 sample, grapes were cultivated in appellation controlled Mukuzani micro-zone was used. S1 sample was made according to the Kakhetian wine making method. White wine RK3 exhibited the highest ability to reduce lipase activity, and lipase unit was equal to 666.08 ± 57.84 per mg enzyme. This wane was made in qvevri and was characterised by noticeably high total polyphenol content and antioxidant activity. The lipase activity with the presence of 10 mg of Orlistat was equal to 487.67 ± 21.142 per mg enzyme.

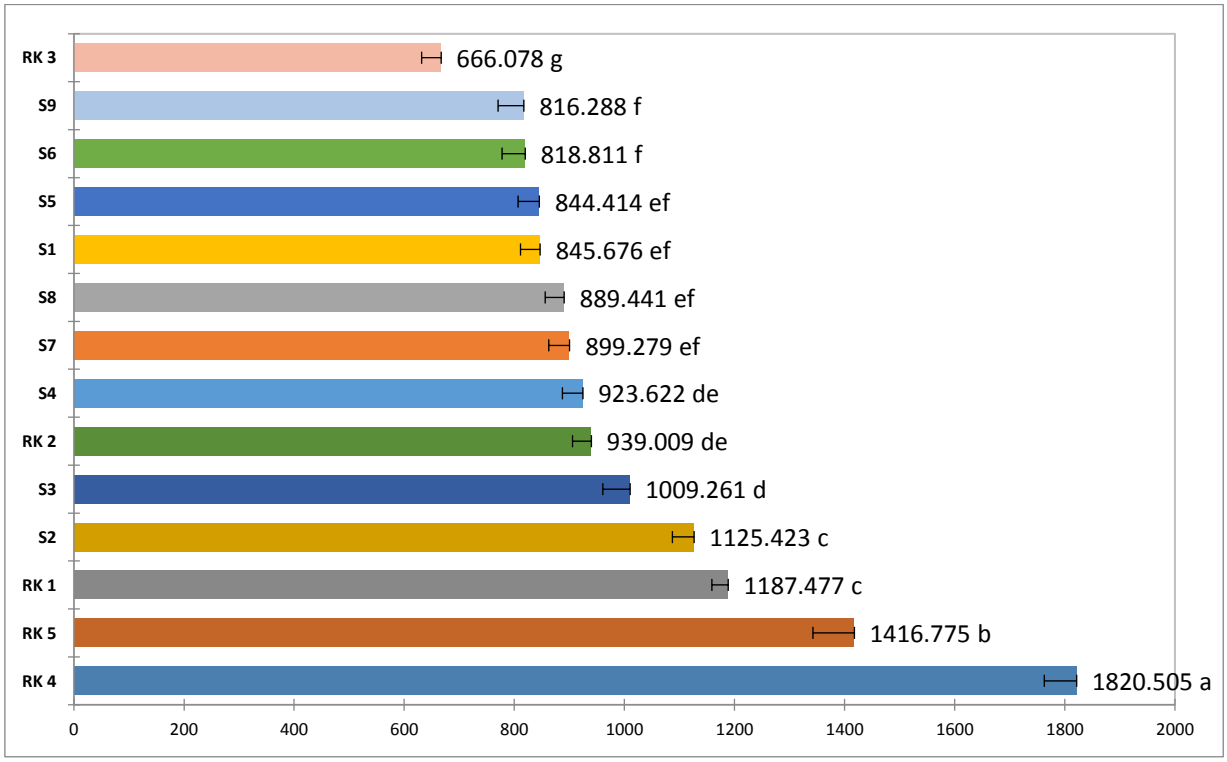

Figure 12. Inhibition effects of different wines on PL activity. Activity values on axis are given in lipase units per mg enzyme. Means of triplicate measurements. Different alphabet letters indicate the significant difference at p < 0.001. S1- Glekhuri, Khasmi Saperavi; S2 -

Matrobela, Saperavi; S3 - Icewine, Guramishvilis Marani, Saperavi; S4 -Mukuzani Valley, Mukuzani (2016); S5 - Mukuzani Valley, Mukuzani (2019); S6 - Rtvelisi, Mukuzani ; S7 - Zurab Tsereteli, Mukuzani; S8- Zhamurashvili's wine, Mukuzani; S9 - Nekresi Estate, Mukuzani; RK1 - Icewine Satrapezo, RK2 – Rkatsiteli, Vine Ponto; RK3 - Mr Rkatsiteli from Gurjaani; RK4 - Vaziani, Rkatsiteli; RK5 - Rkatsiteli;

4.6 Lipase Inhibition Percentage by Different Wine Samples

After quantifying the amount of enzyme that hydrolyses 1.0 micro equivalent of fatty acid from a triglyceride in one hour at pH 7.2 at 37 °C, the inhibition percentage for each wine sample was determined. Calculated data for each wine sample is reflected in Figure 13.

The highest level of lipase inhibition among the given samples was exhibited by the sample RK 3, 65.27% for 2 mL of wine. This white wine sample was made by Kakhetian winemaking method. It is noteworthy, that wines from Mukuzani microzone showed high anti-lipase activity; their lipase inhibitory activity ranged from 54.25 to 59.57% mL^{-} of wine. No significant difference was observed among these samples. Orlistat (10mg) itself showed 75.84% inhibition of lipase activity. The lowest lipase inhibitory activity was shown by the sample RK4. 2 mL of wine sample reduced lipase activity by 9.82%. RK4 belongs to the class of dry white wines made with classic European technology. This sample also contained a low amount of dry extract, TPC and AOA compared to the other samples. The second sample with a low lipase inhibitory activity was also a white wine sample, RK5, with 29.82 inhibition per cent. The amount of TPC in this sample was the lowest compared to other samples - 190.24 mg GAE L^{-1}. At an average rate, white wines made by the classical technology possessed lower anti-lipase activity (26.94% per 2 ml of wine) compared to red wines (53.6% per 2 ml of wine) processed with the same winemaking method. White wine RK1 made with Qvevri technology revealed higher percentage of lipase inhibition (41.18 %) than Saperavi samples (S2, S3) made with the European winemaking method, 44.25 and 50.01%, respectively. No significant difference was observed among the other Saperavi samples, which differed by the winemaking method.

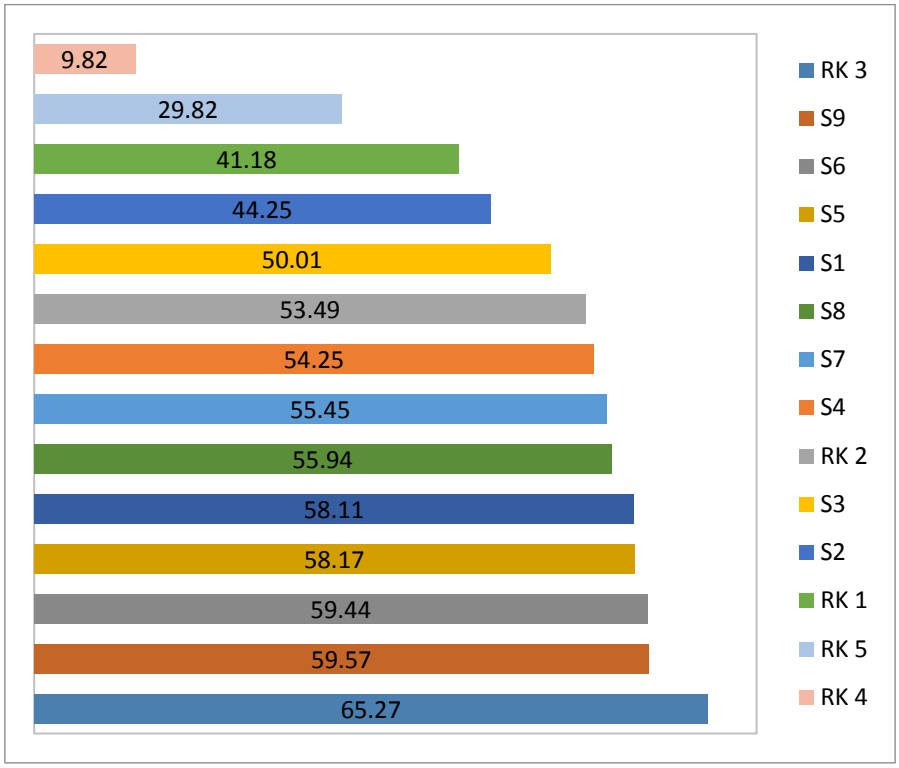

Figure 13. Lipase inhibition percentage by different wine samples. S1- Glekhuri, Khasmi Saperavi; S2 - Matrobela, Saperavi; S3 - Icewine, Guramishvilis Marani, Saperavi; S4 - Mukuzani Valley, Mukuzani (2016); S5 - Mukuzani Valley, Mukuzani (2019); S6 - Rtvelisi, Mukuzani ; S7 - Zurab Tsereteli, Mukuzani; S8- Zhamurashvili's wine, Mukuzani; S9 - Nekresi Estate, Mukuzani; RK1 - Icewine Satrapezo, RK2 – Rkatsiteli, Vine Ponto; RK3 - Mr Rkatsiteli from Gurjaani; RK4 - Vaziani, Rkatsiteli; RK5 - Rkatsiteli;

Although wines produced by the common European method demonstrated lowest inhibition percentage, no significant correlation was found between polyphenol content and anti-lipase activity in wine samples (R^2=0.4407), nor between lipase inhibitory activity and winemaking method. This could be due to the significant difference in the content of individual phenolic compounds in different wines. Because, the lipase inhibitory activity of these compounds differs. In a previously published study, the R^2 correlation value between lipase inhibitory activity and TPC was also found to be low (Gulua et al. 2018).

4.7 Relative Inhibition Percentage in the Presence of 1 mg Inhibitor

As mentioned earlier, 1 mL of double concentrated wine samples was incorporated into the reaction mixture. We also aimed to calculate lipase inhibition percentage per 1 mg dry matter, thus the relative amount of lipase activity and inhibition percentage was calculated (table 7, See Appendix B). From obtained data relative inhibition percentage in the presence of 1 mg inhibitor was calculated.

The highest level of lipase inhibition was shown by the white wine samples (RK 4, RK 3 and RK 5) and no statistically significant differences existed between them. Lipase inhibition percentage displayed by 1 mg of these samples were as follows: 2.04 ± 0.07; 2.03 ± 0.02 and 1.95 + 0.03. These wine samples possessed lower dry matter content compared to the other samples. Overall, 1 mg white wine samples exhibited higher lipase inhibitory activity, then the other samples. The only exception was RK1 wine sample, that belonged to the late harvest wine. This white wine together with the sample S3 (that also belonged to the ice wine category) exhibited the lowest percentage of inhibition (0.36 and 0.25%) due to the high content of the dry extract. No statistically significant difference was found between following wine samples RK2, S9, S1, and S6, they inhibited PL activity on an average 1.26 ± 0.02%. These wines were made in Qvevri according to the Kakhetian winemaking method. The anti-lipase activity of Orlistat® itself calculated per mg was equal to 7.58 %. The lipase inhibition potency by the 1 mg of the rest of the samples ranged between 1.08 – 0.8%.

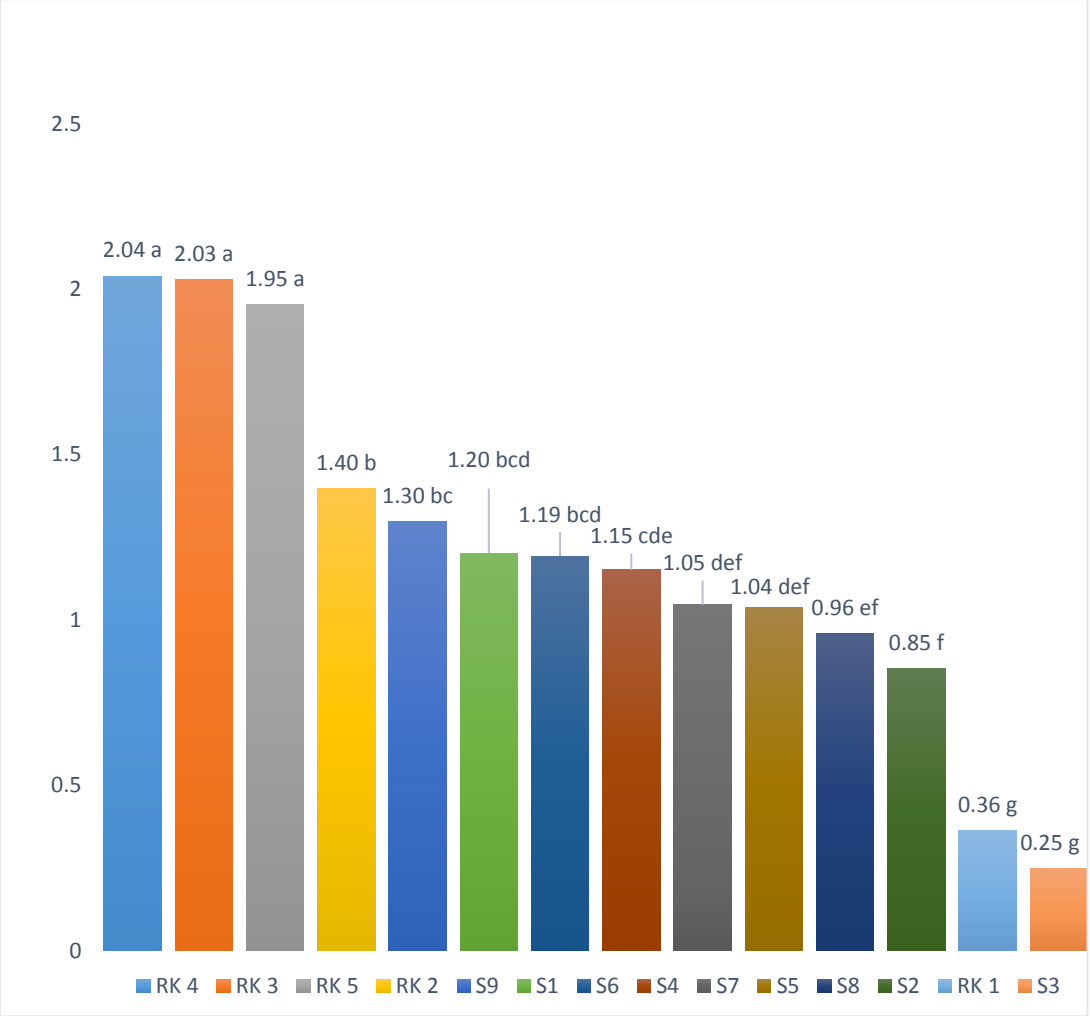

Figure 14. Relative Inhibition % based on 1 mg dry matter of different wine samples. Same alphabet letters indicate no statistically significant difference at p < 0.001. S1- Glekhuri, Khasmi Saperavi; S2 - Matrobela, Saperavi; S3 - Icewine, Guramishvilis Marani, Saperavi; S4 -Mukuzani Valley, Mukuzani (2016); S5 - Mukuzani Valley, Mukuzani (2019); S6 - Rtvelisi, Mukuzani ; S7 - Zurab Tsereteli, Mukuzani; S8- Zhamurashvili's wine, Mukuzani; S9 - Nekresi Estate, Mukuzani; RK1 - Icewine Satrapezo, RK2 – Rkatsiteli, Vine Ponto; RK3 - Mr Rkatsiteli from Gurjaani; RK4 - Vaziani, Rkatsiteli; RK5 - Rkatsiteli;

4.8 Estimation of Wine Quantity to Provide Analogue Inhibition of Orlistat

From obtained results and based on the dry extract content in wine samples, provided 100% 0f dru matter of wine is absorbed by human body, one can calculate the optimal dose (in mL) required to achieve the same inhibition results by wine as the intake of 120mg Orlistat gives. The results are presented in the table 4.

Table 4. Calculated doses of wine samples to achieve same inhibition percentage as 120 mg Orlistat, provided 100% bioavailability

Wine Sample	mL
S2	41.24 a
S3	36.46 ab
S4	33.59 bc
RK 4	33.19 bc
S7	32.86 bc
S1	31.35 bcd
S5	31.32 bcd
S6	30.65 cd
RK 5	28.06 cde
S8	26.41 def
RK 1	25.79 def
RK 2	23.72 ef
S9	22.82 ef
RK 3	22.03 f

Same alphabet letters indicate no statistically significant difference at $p < 0.001$. S1- Glekhuri, Khasmi Saperavi; S2 - Matrobela, Saperavi; S3 - Icewine, Guramishvilis Marani, Saperavi; S4 -Mukuzani Valley, Mukuzani (2016); S5 - Mukuzani Valley, Mukuzani (2019); S6 - Rtvelisi, Mukuzani ; S7 - Zurab Tsereteli, Mukuzani; S8- Zhamurashvili's wine, Mukuzani; S9 - Nekresi Estate, Mukuzani; RK1 - Icewine Satrapezo, RK2 – Rkatsiteli, Vine Ponto; RK3 - Mr Rkatsiteli from Gurjaani; RK4 - Vaziani, Rkatsiteli; RK5 - Rkatsiteli;

Calculated data showed that approximately 21 – 44 mL of wine is needed to achieve same inhibition percentage of PL as Orlistat, only if dry matter digested fully. However, the real quantity of wine with the inhibition value equivalent to 120 mg Orlistat depends on critical variables of wine polyphenols, such as the bioavailability, metabolism, and stability under in vivo conditions (Fernandes et al. 2017). If phenolic substances or their active metabolites are not absorbed in sufficient quantities and in a form that cells can use, they are less likely to have considerable in vivo activity (Stockley et al. 2012).

The majority of the phenolic compounds present in wine are in the form of esters, glycosides, or polymers, which cannot be absorbed in the small intestine. Before absorption, they must be hydrolysed by intestinal enzymes or by the colonic microflora (Scalbert and Williamson, 2000). Phenolic compounds are absorbed into the human body in several different ways. Additionally, many factors can interfere with the integration of different flavonoids and non-flavonoids, such as for example the structure of polyphenols. Other disturbing conditions could be that polyphenols cannot be absorbed via the intestinal barrier, are eliminated in bile, or processed by colonic microbiota of human tissues.

Several studies attempted to evaluate the bioavailability and metabolism of wine polyphenols. Scalbert and Williamson (2000), found that after drinking 750 mL red wine containing 14– 16 mg quercetin, the amount of accounted quercetin as a percentage of consumption was 0.8 %. Donovan et al. (2002)evaluated urinary excretion of catechin metabolites by human volunteers after drinking red. Research findings indicated that humans absorb between 3.0 to 10.3 per cent of the ingested catechins found in red wine. Anthocyanins have been reported to have some of the lowest bioactivity of all the dietary flavonoid subclasses, with bioavailability of less than 1% of the ingested amount (Frank et al. 2003). Manach et al. (2005) suggested, that anthocyanins have bioavailabilities ranging from 2.5 to 18.5 per cent. Later, Fernandes et al. (2012) and Oliveira et al. (2015) developed an in vitro gastric model, proving that after 3 hours of incubation 10% of anthocyanins can be absorbed.

Although review articles indicate the knowledge gap between the bioavailability of wine flavonoids and their health-promoting effects (Fernandes et al. 2012; Stockley et al. 2012), studies conducted by (Gowd et al. 2019) Cardona et al (2005) and indicated that in the small intestine, about 5%–10% of total polyphenolic compounds are absorbed, consequently subjected to more extensive processing. Remaining polyphenols are likely to be collected in the large intestine and eliminated in the stool. Based on this, we can assume that the intake

of an average 275 ml of wine (minimum of 105 – maximum of 440 mL could be enough to inhibit PL activity as much as 120 mg Orlistat. However, only inhibition potency itself is a relatively uninformative quantity (Brooks et al. 2012). In order to rank new chemicals regarding their inhibitory potency, it is essential to define their mode of inhibition (competitive, uncompetitive, non-competitive, or mixed) and kinetics of inhibition in the wine samples.

4.9 Kinetics and Inhibition by Wine and Its Phenolic Fractions

To study the kinetics and mechanism of PL inhibition by wine, Mukuzani wine (sample #S9) was chosen. Selected wine was produced from Saperavi and made with Kakhetian winemaking method. Based on the obtained results this wine possessed noticeably higher lipase inhibitory activity compared to the other samples. The TPC and AOA of the same sample were greater too. The same wine sample was fractionated into different phenolic compounds and their mechanism and kinetics of inhibition towards PL were also evaluated.

After extraction aqueous and organic fractions were obtained. Total phenolic content in the fractionated parts was measured, which were as follows 2078±89.75 and 791.22±31.659 $\mu g \cdot mL^{-1}$ Gallic acid equivalent. The total phenolic content in chosen wine sample itself was equal to 2965.312±67.152 $\mu g \cdot mL^{-1}$ GAE. Acceptable margin of error indicated a successful fractionation process.

4.7.1 Kinetic and mechanism of Pancreatic Lipase Inhibition by wine

The mode of PL inhibition exhibited by the Lineweaver-Burk plots of wine was found to be mixed-inhibition (Figure 15), which showed that wine was able to bind to the enzyme (PL) and also to the complex formed between the enzyme and the substrate (PL-Olive oil complex) (*29*).

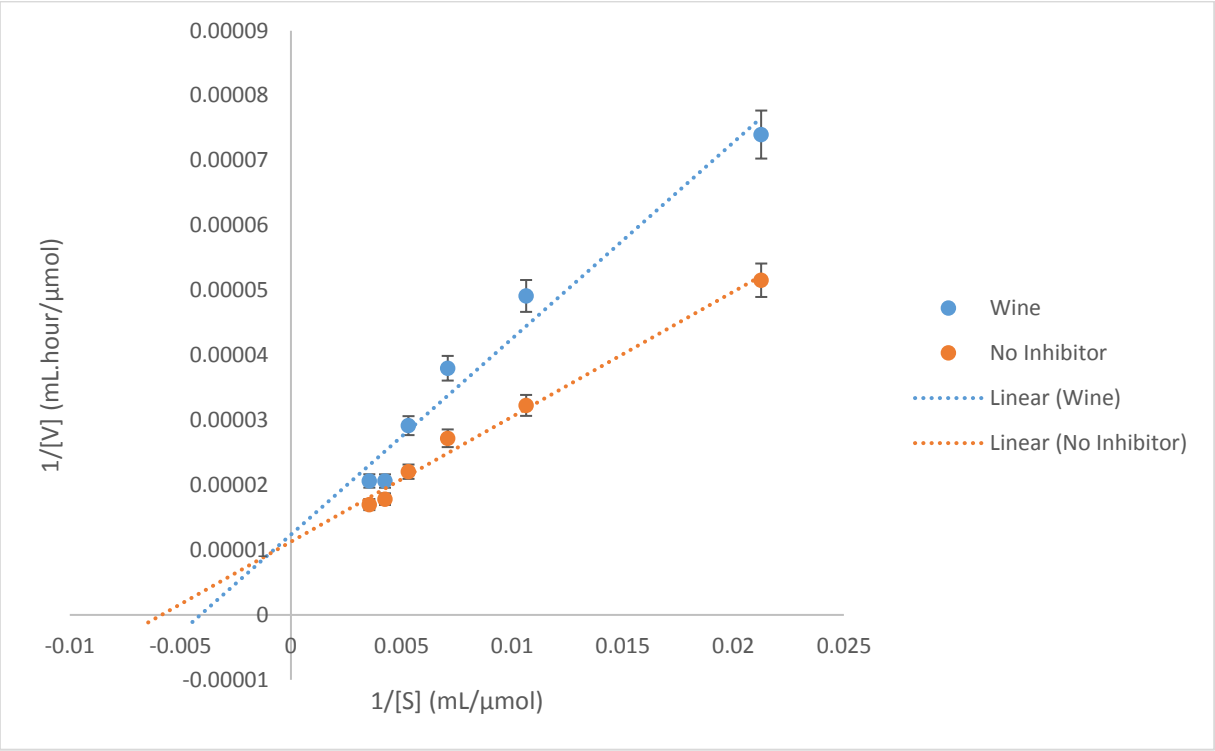

Figure 15. Lineweaver-Burk plots of pancreatic lipase activity with olive oil as substrate with and without wine

Based on MOI, the overall inhibition reaction mechanism of mixed inhibition featured by wine is shown on the scheme (1). Based on this reaction mechanism, the inhibitor is capable to bind to both - the free enzyme and the enzyme-substrate complex. K_{i1} is the inhibition constant for binding of wine to the PL and K_{i2} is the inhibition constant for binding of wine to the PL -Olive Oil complex. This interaction promotes a decrease of V_{max} (maximum velocity of the enzyme) and an increase in K_m in the presence of an inhibitor (*29*).

$$\begin{array}{ccccc} E + S & \leftrightarrow & ES & \rightarrow & E + P \\ + & & + & & \\ I & & I & & \\ K_{i1} \updownarrow & & K_{i2} \updownarrow & & \\ EI + S & \leftrightarrow & EIS & & \end{array}$$

Scheme 1. Scheme of a Reversible Linear Mixed Inhibition. [E]: enzyme; [S]: substrate; [I]: inhibitor; [P]: product, [K_{i1}] & [K_{i2}]: Inhibition constants. Wine as the inhibitor (I) can interact with the free enzyme (E) to form the complex enzyme/inhibitor (EI), as well as with the enzyme/substrate complex (ES) to form EIS complex.

Overall PL inhibition reaction kinetics can be described characterised by the reciprocal data equation (eq. 5) (*26*), which shows that due to the inhibitory effect given by wine the slope of K_m/ V_{max} is decreased by a factor of $(1+[I]/K_{ia})$.

$$\frac{1}{V} = \left(1 + \frac{[I]}{K_{i1}}\right)\frac{K_M}{V_{max}}\frac{1}{[S]} + \frac{\left(1+\frac{[I]}{K_{i2}}\right)}{V_{max}} \qquad /5/$$

where [V] stands for the reaction velocity, [V_{max}] – the maximum reaction velocity, [K_m] – Michaelis-Menten constant, [S] - substrate concentration, [K_{i1}] – inhibition constant for free enzyme, [K_{i2}] – inhibition constant for enzyme-substrate complex.

By comparing Eqs. 3 and 4, following equations can be used to calculate apparent values of maximum reaction velocity and Michaelis-Menten constant

$$V_{max,app} = \frac{V_{max}}{1+\frac{[I]}{K_{i2}}} \qquad /6/$$

$$K_{m,app} = K_m \frac{1+\frac{[I]}{k_{i1}}}{1+\frac{[I]}{k_{i2}}} \qquad /7/$$

where [V_{max}] stands for the maximum reaction velocity, [$V_{max,\,app}$] - for the maximum reaction velocity in inhibition study, [K_m] – Michaelis-Menten constant, [$K_{m,\,app}$] – Michaelis-Menten constant in inhibition study, [S] - substrate concentration, [I] - inhibitor concentration, [K_{i1}] – inhibition constant of free enzyme, [K_{i2}] – inhibition constant of enzyme-substrate complex.

Calculated values of kinetic parameters are shown in Table 5. As seen from Table 5, the kinetic parameters of reaction without inhibitor (control) were as follows: Michaelis-Menten

constant (K_m) was equal to 170.901±7.544 μmol·mL^{-1} and the maximum reaction rate (V_{max}) was equal to 88735±4036.741 μmol·mL^{-1}·hour^{-1}. In the presence of wine, the value of V_{max} decreased and was equal to 80875.4 ± 3489.754 μmol·mL^{-1}·hour^{-1}. This meant that wine was proficient to prevent catalysis regardless of whether the inhibitor was attached to the free enzyme or to the enzyme-substrate complex (Scheme 1). The value of Michaelis Menten constant (K_m) increased up to 244.329± 10.214 μmol·mL^{-1}. A larger value of K_m shows a weak binding of a substrate to an enzyme (K. Buchholz, Kasche, and Bornscheuer 2012).

*Table 5. **Kinetic parameters of inhibition reaction by wine and wine fractions***

Sample	Linear Equation	R^2	Km μmol·mL^{-1}	Vm μmol · mL^{-1} · hour^{-1}	Ki (1) μmol·mL^{-1}	Ki (2) μmol·mL^{-1}
No inhibitor (Control)	y = 0.0019259707x + 0.0000112695	0.9879	170.901 ± 7.544 b	88735.08 ± 4036.741 a	N/A	N/A
Wine	y = 0.0030210571x + 0.0000123647	0.9637	244.329 ± 10.214 a	80875.4 ± 3489.754 b	40.556 ± 1.932	179.361 ± 8.678
Ethyl acetate fraction	y = 0.0020081973x + 0.0000238395	0.9186	84.238 ±3.455 c	41947.19 ± 2001.395c	4.521± 0.209	N/A
Aqueous fraction	y = 0.0018547013x + 0.0000254384	0.9111	72.91± 3.333 d	39310.65 ± 1687.53 c	1.978 ± 0.086	N/A

Mean values in the column followed by the identical letters are NOT significantly different ($P < 0.05$).

Based on MOI, two values of inhibition constants (Ki) were obtained. K_{i1} is the inhibition constant for binding wine to the PL and shows the concentration of wine that is needed to reduce affinity of lipids to the PL by half. K_{i2} is the inhibition constant for binding wine to the PL -Olive Oil complex and indicates the concentration of wine to reduce PL activity twice. The values of K_{i1} and K_{i2} were calculated by using Eqs. 6 and 7, where [I] refers to the

concentration of total phenolic compounds μmol·mL^{-1} in wine since we believe that the inhibitory effect of wine is caused due to the polyphenol content. The results showed that the value of K_{i1} was smaller than the value of K_{i2}, 40.556 ± 1.932 and 179.361 ± 8.678 μmol·mL^{-1}, respectively. This indicated that the affinity of wine to bind to the free enzyme (PL) was higher than the binding affinity to enzyme-substrate complex, which makes the inhibitory effect stronger. According to the Nomenclature Committee of the International Union of Biochemistry (NC-IUB), we can entitle this case as predominantly competitive inhibition, since $K_{i1} < K_{i2}$ (Buchholz et al., 2012).

A similar study was conducted by Gu et al., (2011). They assessed in vitro inhibitory effects of cocoa extracts and procyanidins against digestive enzymes. This study showed that the regular cocoa extract, procyanidin pentamer, and decamer reduced the V_{max} and increased K_m of PL, by that means suggesting a mixed-type inhibition. Another study evaluated the inhibitory effect of some plant extracts on PL and a similar inhibition mode was observed in *Levisticum officinale* methanolic extract against PL (Gholamhoseinian, Shahouzehi, and Sharifi-fa 2010). Similar results were showed by Muhd Rodhi et al., (2020) when evaluating kinetics behaviour of pancreatic lipase inhibition by the crude leaves extract of Aquilaria species. Based on the finding the presence of Gallic acid and quercetin in extracts had influenced the inhibition of PL and provided highest percentage of inhibition.

4.7.2 Study of Kinetics and Mechanism of Pancreatic Lipase Inhibition by Phenolic Fractions of Wine

Wine contains enormous and various classes of phenolic compounds (Giovinazzo, Carluccio, and Grieco 2019). The inhibitory activity of wine can be influenced by them. In order to determine the inhibition potency of different phenolic fractions, liquid-liquid extraction was carried out. Two different phenolic extracts were obtained. According to Roussis et al. (2005), the aqueous phase mainly contains anthocyanins and polymerised phenolic compounds. The ethyl acetate fraction, on the other hand, is rich in flavanols, flavonols, and phenolic acid.

The Lineweaver-Burk plots of pancreatic lipase activity with aqueous and ethyl acetate fractions (Fig. 16 and 17, respectively) showed a pattern of parallel lines. As it can be seen from the figures, inhibitors changed the intercept of the ordinate. The formation of the parallel lines on the plot indicates an uncompetitive inhibition mechanism.

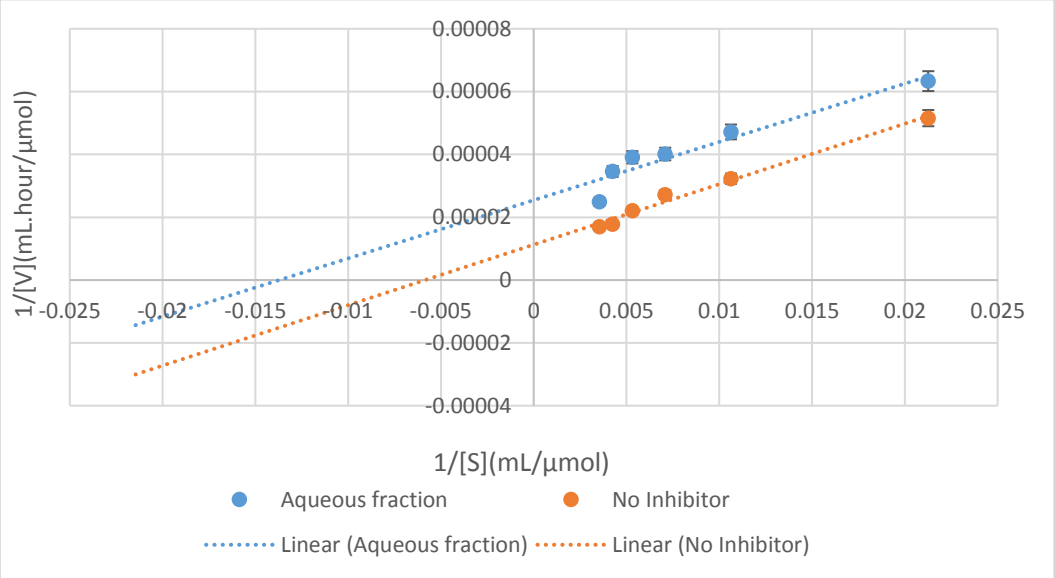

Figure 16. Lineweaver-Burk plots of pancreatic lipase activity with olive oil as substrate with and without aqueous fraction.

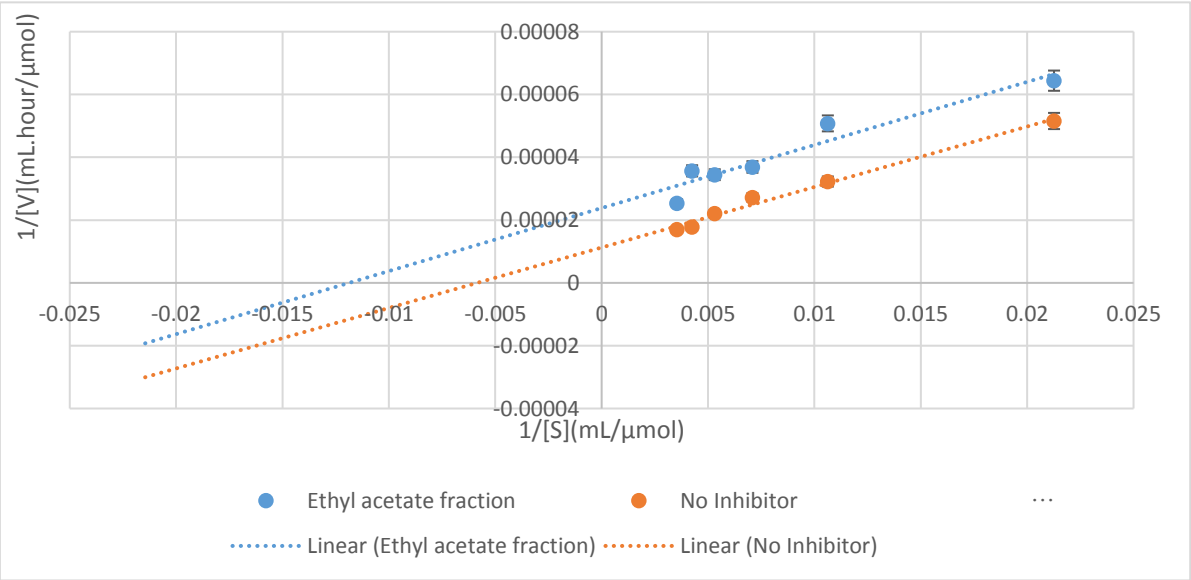

Figure 17. Lineweaver-Burk plots of pancreatic lipase activity with olive oil as substrate with and without ethyl acetate fraction.

The overall inhibition reaction mechanism of uncompetitive inhibition is demonstrated at scheme 2.

$$\begin{array}{c} E + S \leftrightarrow ES \rightarrow E + P \\ + \\ I \\ K_i \updownarrow \\ EIS \end{array}$$

Scheme 2. Scheme of uncompetitive inhibition. [E]: enzyme; [S]: substrate; [I]: inhibitor; [P]: product; [K_i] inhibition constant. Wine fractions as inhibitors (I) can interact with the enzyme/substrate complex (ES) to form the complex (EIS).

Results showed that wine phenolic fractions were able to bind to the complex formed between the enzyme and the substrate (PL - olive oil complex) (scheme2). Based on MOI, following equations were used to calculate kinetic parameters:

$$V_{max,app} = \frac{V_{max}}{1+\frac{[I]}{K_i}} \quad /8/$$

$$K_{m,app} = \frac{K_m}{1+\frac{[I]}{K_i}} \quad /9/$$

where [V_{max}] stands for the maximum reaction velocity, [$V_{max,\,app}$] - for the maximum reaction velocity in inhibition study, [K_m] – Michaelis-Menten constant, [$K_{m,\,app}$] – Michaelis-Menten constant in inhibition study, [S] - substrate concentration, [I] - inhibitor concentration, [K_i] – inhibition constant of enzyme-substrate complex.

As seen from Table 5, here the values of the apparent $V_{max,app}$ and $K_{m,app}$ decreased. $K_{m,app}$ of ethyl acetate fraction was equal to 84.238 ±3.455.238 μmol·mL^{-1} and $V_{max,app}$ was equal to

41.947.19± 2001.395 μmol mL^{-1}·$hour^{-1}$. The value of $K_{m,app}$ for aqueous fraction was found to be 72.91 ± 3.33 μmol·mL^{-1} and $V_{max,app}$ -39310.65 ± 1687.53 μmol·mL^{-1}·$hour^{-1}$. As observed, uncompetitive inhibitors decreased both kinetic parameters. As seen from scheme 2, uncompetitive inhibitors only block processes beyond ES formation. For this reason, one might expect the reduction of only maximum reaction velocity ($V_{max,app}$). However, after binding to ES and stabilizing the complex, the inhibitor makes more difficult for a substrate to dissociate or be converted into a product. Therefore, enzyme affinity for a substrate is increased, which subsequently causes the reduction of K_m. This mode of action is an attractive approach for drug design as the inhibitors bind to the enzyme target only when the target is active and substrate present (Dougall and Unitt 2015). According to Cornish-Bowden (1986), uncompetitive inhibition are ideal for disrupting enzyme targets within metabolic pathways. The inhibition constant for ethyl acetate and aqueous fractions were 4.521±0.209 and 1.978±0.086 μmol·mL^{-1}, respectively. A small value of Ki indicates a stronger inhibitory effect because the inhibitor bounds tightly (Cornish-Bowden 2012). Due to the uncompetitive inhibition, these kinetic parameters were decreased by the same factor.

Moreno-Córdova et al., (2020) evaluated the inhibitory activity of selected phenolic compounds and showed that Gallotannins are uncompetitive inhibitors of pancreatic lipase activity. However, due to the huge number of molecular structures of phenolic compounds, the specific mechanism of action varies significantly, even between related compounds (Glisan et al. 2017; Wang et al. 2014).

4.7.3 Evaluation of Combined Inhibition Effects of Phenolic Extracts on the PL

In this study, we also wanted to evaluate the combined effect of two inhibitors on the PL activity. Overall, in the presence of two or more inhibitors of various types, the combined effect on the enzyme activity obeying Michaelis-Menten kinetics may lead to synergism,

summation, or antagonism (Chou and Talaly, 1977). The following generalised equations were used to determine the combined effect of two different inhibitors:

Summation:

$$\frac{1}{v_{1,2}} = \frac{1}{v_1} + \frac{1}{v_2} - \frac{1}{v_0} \qquad /10/$$

Synergism:

$$\frac{1}{v_{1,2}} > \frac{1}{v_1} + \frac{1}{v_2} - \frac{1}{v_0} \qquad /11/$$

Antagonism:

$$\frac{1}{v_{1,2}} < \frac{1}{v_1} + \frac{1}{v_2} - \frac{1}{v_0} \qquad /12/$$

where $v_{1,2} = v_1 v_2 / v_0$ and v_0 denotes the velocity in the absence of inhibitor, v_1- the velocity in the presence of inhibitor 1, v_2- the velocity in the presence of inhibitor 2.

The derivation of summation is based on the assumption that each enzyme species can combine with no more than one of the inhibitors. Deviations from this equality define synergism when the value of the left side is greater than the right or antagonism, when the value of the left side is smaller than the right. As we can see from the equations, the knowledge of the kinetic constants, such as kinetic constants for substrates and inhibitors, is not required. Additionally, few measurements are needed to obtain these quantitative descriptions.

In our case, we found that the value of $\frac{1}{v_{1,2}}$ (0.0000538 ± 0. 0.0000024 mL·hour·μmol^{-1}) was greater than the value of $\frac{1}{v_1} + \frac{1}{v_2} - \frac{1}{v_0}$ (0.0000380 ± 0.00000271 mL·hour·μmol^{-1}). This data indicated that the ethyl acetate and aqueous fractions had a synergistic effect on the activity of PL. These inhibitors can also be called mutually nonexclusive inhibitors, because they act independently on a single target (Chou and Talaly, 1977).

4.10 The link between SDG and research findings

As discussed in the section 2.3.2 prevalence of obesity undermines the achievement of SDG 3. Experimental data showed that wine is able to attenuate PL activity in vitro. By reducing the consequences of high-fat diets in humans, wine can be regarded as a promising source to fight adiposity. Hence, will be useful to achieve SDG 3. According to the critical review published by (Bennett et al. 2020), although there has been significant progress on many SDG 3 targets, however target 3.4 – "*reduce by one third premature mortality from non-communicable diseases (NCDs) through prevention and treatment and promote mental health and wellbeing*" – is severely lagging behind. Since NCDs are strongly associated with obesity and overweight, tackling adiposity is at the heart of achieving the SDG 3.4.

Additionally, research findings are linked to SDG 2 "*End hunger, achieve food security and improved nutrition and promote sustainable agriculture.*" Wine has been consumed for millennia all over the world, and in addition to pure hedonistic enjoyment, many papers have been written about its nutritional benefits. Over the last decades, researchers have investigated the health advantages of moderate wine consumption. However, mostly the benefits of red wine have been studied, due to the higher content of polyphenols, antioxidants and resveratrol. These substances are found in the grape skins, which, compared to the white wine manufacturing, are more significant for red wine production in the case of European winemaking technology (Golan, Gepner, and Shai 2019). Research findings show that white Qvevri wines contain comparable number of polyphenols to red wines made with European technology. Furthermore, we also defined obesity in forms of malnutrition, in this case wine can be useful adjunct to solve both obesity and malnutrition.

Due to the synergies and trade-offs within SDGs, several goals and targets need to be considered when targeting obesity with lipase inhibitory activity of wine. Firstly, target 3.5 deserves special attention, which aims to "*strengthen the prevention and treatment of substance abuse, including narcotic drug abuse and harmful use of alcohol*". This target

implies that there is a healthy level of alcohol use, but it also shows a clear need of quality education (SDG 4) and responsible consumption (SDG12). Higher awareness may affect healthy and sustainable drink choices and responsible consumption. Consistently, SDG 10, with the aim of reduction inequalities is also important, since inequalities cause disparities in income, health, food and education access.

SDG 12 is also interesting from the sustainable production point of view. Excessive use of water contributes to the global water stress. Wineries create significant amounts of wastewater that are generated at different stages of winemaking (Bolzonella et al. 2019; Conradie, Sigge, and Cloete 2016), and Implementation of target 12.8 "*ensure that people everywhere have the relevant information and awareness for sustainable development and lifestyles in harmony with nature* " and target 12.4 - "*achieve the environmentally sound management of chemicals and all wastes throughout their life cycle, in accordance with agreed international frameworks, and significantly reduce their release to air, water and soil in order to minimize their adverse impacts on human health and the environment*" will contribute sustainable winemaking process.

Additionally, throughout winemaking clean water (SDG 6) is needed. From this point of view target 6.4 is also interesting to note, which aims to "substantially increase water-use efficiency across all sectors and ensure sustainable withdrawals and supply of freshwater to address water scarcity and substantially reduce the number of people suffering from water scarcity". This target is connected to responsible production as well since achieving it is really hard without increasing water-use efficiency.

Besides all the SDGs mentioned above, successful implementation of following goals is important to manage obesity with the help of wine.

SDG 1 - "No Poverty" – poverty limits access to adequate food intake and makes it difficult to reach nutritional recommendations. People on low income are price sensitive, thus they will prefer purchase other goods than wine.

SDG 13. Climate action - Climate change affects global food production and food security as well as access to fresh water resources. Climate is one of the most important influencing elements in grape and wine production (Fraga et al. 2014), impacting the suitability of specific grape types for a given region, as well as the type and quality of wine produced (Fraga et al. 2016; Gladstones 2016). Wine composition is greatly influenced by the mesoclimate and the microclimate and in order to create high-quality wines climate-soil-variety balance must be maintained (Grzeskowiak et al. 2013). Because the presence of phenolic compounds in wine is greatly influenced by temperature, an increase in average temperatures will affect their presence in wine areas, therefore will affect the grape quality and subsequently will decrease the anti-lipase activity of wines.

Above mentioned climate-soil-variety equilibrium also leads to SDG 15 (Life on land). Change in land use causes soil degradation while reducing biodiversity and food production and decreasing access to fresh water.

Another development goal "Peace and justice" (SDG 16) is also interested from the topic's point of view. War causes malnutrition and death due to inadequate/insecure food supplies and reduced access to food. This itself makes life hard on Land. Economic growth (SDG 8) could be also another interesting goal, since economic transformation may provide increased nutrition security and sustainable agriculture.

Last but not least, SDG 17, "Partnerships for goals", deserves the attention. Enhancing the global partnership with Georgian wine cellars or wineries with the governments could be interesting. Providing access to wines made with Kakhetian winemaking technology and

explaining to the vintners all the stages of Georgian winemaking tradition is important. After all, it would be easy to fight against obesity together.

Although research findings are primarily linked with SGD 2 and 3, it is important to note that the implementation of several SDGs is also needed to use wine against obesity. In addition, some SDGs play a prominent role in defining the quality characteristics of wine. Hence, all SDGs discussed above need to act like cogwheels.

5. Conclusion

The present study investigated the inhibitory potential of Georgian wine against PL. Additionally it identified the mode of inhibition and determined kinetic parameters of Mukuzani wine and its phenolic extracts. Research Outcomes provide the first evidence that red wine is a potent inhibitor of pancreatic lipase with mixed-type inhibitory activity.

Kinetic analysis showed that wine produced according to the Kakhetian method inhibited PL activity in a mixed-mode (competitive-uncompetitive). Overall, results indicated that the affinity of wine to bind to the free enzyme (PL) was higher than the binding affinity to enzyme-substrate complex, which makes the inhibitory effect stronger.

Ethyl acetate fraction and aqueous fraction uncompetitively inhibited PL activity. The ethyl acetate fraction and aqueous fraction had synergistic effects on a single target enzyme (PL) and were considered as mutually nonexclusive inhibitors. This study demonstrated that red wine made with the Kakhetian winemaking method can inhibit pancreatic lipase in vitro and may play a role in body weight management.

In white wines produced by the Kakhetian method, antioxidant activity and TPC is significantly higher than in the white wines prepared by the common European method.

No significant correlation was found between polyphenol content and anti-lipase activity in wine samples, nor between lipase inhibitory activity and winemaking method.

Overall, it can be concluded that most of the wine samples we have examined, are potential inhibitors of PL. Regarding the results, we may conclude that the wines on the Georgian

market made from local cultivars, i.e. Saperavi (red) and Rkatsiteli (white), are characterised by noticeably high anti-lipase and antioxidant activity and high polyphenol content.

The inhibitor activity and kinetic parameters determined from wine are expected to benefit in controlling obesity and problems associated with excess weight.

Further research is needed to define the inhibitory effect of individual polyphenols towards PL and elucidate the anti-obesity effect of Georgian wines *in vivo*.

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Appendices

Appendix A.

Table 6. Proximate chemical composition and AOA of different wine samples

Wine Code	Titratable Acidity g · L^{-1}	Total dry extract g · L^{-1}	Total dry extract g · L^{-1}	Total Polyphenol content mg Gallic acid equivalent L^{-1}	Antioxidant activity mg Ascorbic Acid equivalent L^{-1}
S1	6.588 ± 0.154	24.34 ± 0.02	24.34 ± 0.02	2734.959 ± 59.002 bc	3397.031 ± 194.837 cd
S2	5.776 ± 0.054	25.88 ± 0.07	25.88 ± 0.07	3482.927 ± 136.204 a	4160.465 ± 126.339 b
S3	5.254 ± 0.004	100.54 ± 0.06	100.54 ± 0.06	1828.455 ± 28.455 e	1921.397 ± 119.724 f
S4	7.167 ± 0.0435	25.92 ± 0.02	25.92 ± 0.02	2930.081 ± 74.809 b	3371.412 ± 240.218 cd
S5	6.984 ± 0.232	30.3 ± 0.01	30.3 ± 0.01	3572.358 ± 153.111 a	4729.199 ± 88.162 a
S6	7.119 ± 0.301	25.80 ± 0.12	25.80 ± 0.12	2810.840 ± 297.486 bc	3145.968 ± 113.186 cd
S7	7.15 ± 0.362	26.7 ± 0.09	26.7 ± 0.09	2415.176 ± 19.163 cd	3012.751 ± 119.724 d
S8	8.413 ± 0.381	29.38 ± 0.07	29.38 ± 0.07	2813.550 ± 49.823 bc	3299.678 ± 88.152 cd
S9	6.625 ± 0.002	30.70 ± 0.01	30.70 ± 0.01	2965.312 ± 67.152 b	3494.381 ± 94.199 c
RK 1	7.455 ± 0.032	97.24 ± 0.04	97.24 ± 0.04	149.594 ± 8.13 g	210.073 ± 28.984 g
RK 2	7.932 ± 0.055	27.48 ± 0.01	27.48 ± 0.01	2515.477 ± 97.561 de	2413.275 ± 43.476 e
RK 3	4.961 ± 0.057	20.36 ± 0.02	20.36 ± 0.02	1572.358 ± 56.912 e	1788.181 ± 69.123 f
RK 4	4. 909 ± 0.118	13.46 ± 0.15	13.46 ± 0.15	489.577 ± 36.112 f	179.330 ± 50.722 g
RK 5	6.897 ± 0.226	16.62 ± 0.18	16.62 ± 0.18	190.244 ± 8.13 g	199.825 ± 43.476 g

Means ± standard deviation (SD), different alphabet letters in the same column indicate the significant difference at $p < 0.05$. *S1- Glekhuri, Khasmi Saperavi; S2 - Matrobela, Saperavi; S3 - Icewine, Guramishvilis Marani, Saperavi; S4 -Mukuzani Valley, Mukuzani (2016); S5 - Mukuzani Valley, Mukuzani (2019); S6 - Rtvelisi, Mukuzani ; S7 - Zurab Tsereteli, Mukuzani; S8- Zhamurashvili's wine, Mukuzani; S9 - Nekresi Estate, Mukuzani; RK1 - Icewine Satrapezo, RK2 – Rkatsiteli, Vine Ponto; RK3 - Mr Rkatsiteli from Gurjaani; RK4 - Vaziani, Rkatsiteli; RK5 - Rkatsiteli;*

Appendix B

Table 7. Relative activity and inhibition of lipase in the presence and absence of various inhibitors

Inhibitor	Relative units of lipase by 1mL wine per 1mg enzyme	Relative inhibition percentage by 1 mL of wine	Relative inhibition percentage by 1 mg inhibitor	Effect of 1 mg inhibitor as the per cent of 1 mg Orlistat inhibition value
N/A (Blank)	2018.77 ± 90. 45	N/A	N/A	N/A
Orlistat	487.67 ± 21.142	75.84	7.58	100
S1	1432.23 ± 71.62 fg	29.05	1.19 bcd	15.73975
S2	1572.1 ± 78.61 cd	22.13	0.86 f	11.27306
S3	1514.02 ±75.70 de	25.00	0.25 g	3.279118
S4	1471.2 ± 73.56 ef	27.12	1.05 cde	13.79824
S5	1431.59 ± 71.58 fg	29.09	0.96 def	12.65734
S6	1418.79 ± 70.94 fg	29.72	1.15 bcd	15.18911
S7	1459.03 ± 72.95 ef	27.73	1.04 def	13.69288
S8	1454.11 ± 59.36 ef	27.97	0.95 ef	12.55319
S9	1417.53 ± 57.87 fg	29.78	1.3 bc	17.13331
RK 1	1603.13 ± 80.16 c	20.59	0.36 g	4.785912
RK 2	1478.89 ± 60.38 ef	26.74	1.4 b	18.41172
RK 3	1359.95 ± 68 g	32.63	2.03 a	26.75815
RK 4	1919.64 ± 95.98 a	4.91	2.04 a	26.89573
RK 5	1717.77 ± 85.89 b	14.91	1.95 a	25.74853

Means of triplicate measurements ± standard deviation (SD) in the same column with different alphabet letters indicate a significant difference at $p < 0.001$. S1- Glekhuri, Khasmi Saperavi; S2 - Matrobela, Saperavi; S3 - Icewine, Guramishvilis Marani, Saperavi; S4 - Mukuzani Valley, Mukuzani (2016); S5 - Mukuzani Valley, Mukuzani (2019); S6 - Rtvelisi, Mukuzani ; S7 - Zurab Tsereteli, Mukuzani; S8- Zhamurashvili's wine, Mukuzani; S9 -

Nekresi Estate, Mukuzani; RK1 - Icewine Satrapezo, RK2 – Rkatsiteli, Vine Ponto; RK3 - Mr Rkatsiteli from Gurjaani; RK4 - Vaziani, Rkatsiteli; RK5 - Rkatsiteli;