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# EXTRACTION AND CHARACTERIZATION OF ENZYMATIC ACTIVITY OF DROMEDARY LIPASES FROM SOUF REGION (ALGERIA)

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**Abstract:** This study aims to extract lipase from four organs of camels in the "Targuie" population; namely: pancreas, abomasum, rumen, and bump fat. The presence of this enzyme was confirmed in the mentioned organs by infrared spectroscopy, and the measurement was carried out on the lipase activity of various extracts using a spectrophotometer. The physicochemical properties of the abomasum, pancreas, and rumen revealed interesting values of proteins, total fat, and humidity, respectively as follows: pancreas. In addition, lipid biochemical analyses showed elevated cholesterol levels of 125.48 mg/dl, triglycerides of 30.45 mg/dl, high-density lipoproteins (HDL) of 48.94 mg/dl, lipoproteins low density (LDL) of 110.2 mg /dl, while the fat content was estimated at 75%. Analysis of the results of the infrared spectra confirmed the presence of lipase with values similar to those of the reference spectrum of the lipase. In addition, the determination of the lipase activity gave the following values: the pancreas 0.1359 IU/g, the stomach 0.1306 IU/g, the fat 0.2306 IU/g, the rumen 0.1508 IU/g, these results show that fat contains the highest amount of lipase activity. Through this study, the mysterious animal: the dromedary; was explored differently for the first time by looking for enzymes (lipase) with interesting catalytic activity.

**Key Words:** Algeria, camel, enzymatic activity, infrared spectroscopy, lipase

## INTRODUCTION

Sahara, which is the largest of the deserts, is characterized by edapho-climatic conditions very restricting the spontaneous survival of living beings. Nevertheless, this ecosystem remains a living environment provided with a particular plant cover, adapted to the harshest desert conditions, characterized by high temperatures and low rainfall (Aichouni, 2011).

The dromedary occupies a primordial place because this animal has great capacities to better manage the low density and the low nutritional value of the vegetation in these areas. The dromedary is a sober, rustic animal perfectly adapted to the desert and hot climate. It has physiological and biochemical peculiarities that allow it to fight against environmental constraints (strong nycthemeral thermal difference, low nutritional value, and dispersion of food resources) (Benromdhane *et al.*, 2003).

The organs of the camel, especially the fat from the hump, are used to prepare many dishes in different countries in Asia and North Africa (Sbihi *et al.*, 2013). In Morocco, the fat from the hump of the molten dromedary is consumed fresh alone or combined with aromatic and medicinal plants and the local population recognizes its therapeutic properties (food, massage) (Foughalia, 2017).

Lipases are ubiquitous enzymes that are found in lower organisms such as bacteria, fungi, or yeast as well as in higher organisms such as plants and animals. They form a class of enzymes that are heterogeneous by their origin, whether animal, plant, or microbial, which further increases their potential. They are capable of catalyzing the hydrolysis of glyceride esters in an aqueous medium and the synthesis of esters in a non-aqueous medium (Reis *et al.*, 2008).

Lipases are often regarded as one of the most important and interesting classes of enzymes in the industrial world. This interest comes mainly from the fact, on the one hand, that lipases have atypical catalytic properties and that, on the other hand, the technologies to be used to produce them are relatively simple. Most lipases are stable in many organic solvents and do not require a co-factor to be active. They can be used as a hydrolase or as a catalyst in organic synthesis. Their fields of application are therefore very wide and varied, they can therefore be used in standard organic chemistry to hydrolyze esters on "sensitive" molecules. They are used in the medicinal and pharmaceutical field or the food industry, as well as for the biodiesel industry, cosmetics, and the detergent industry (Fickers *et al.*, 2008).

Very few works have been carried out within the framework of the characterization of dromedary lipases and no study has been published about the zone: Algerian Sahara. This work is part of the development of bio-resources of animal origin, it concerns the extraction of lipase from different organs of the camel and the measurement of its enzymatic activity.

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## MATERIAL AND METHODS

### Biological material

The samples used in this study were provided by the Royal Slaughterhouse, of the wilaya of El-Oued: samples of the fat from the hump; the abomasum, the pancreas, and the rumen of male dromedaries from the Targuie population (Figure 1).

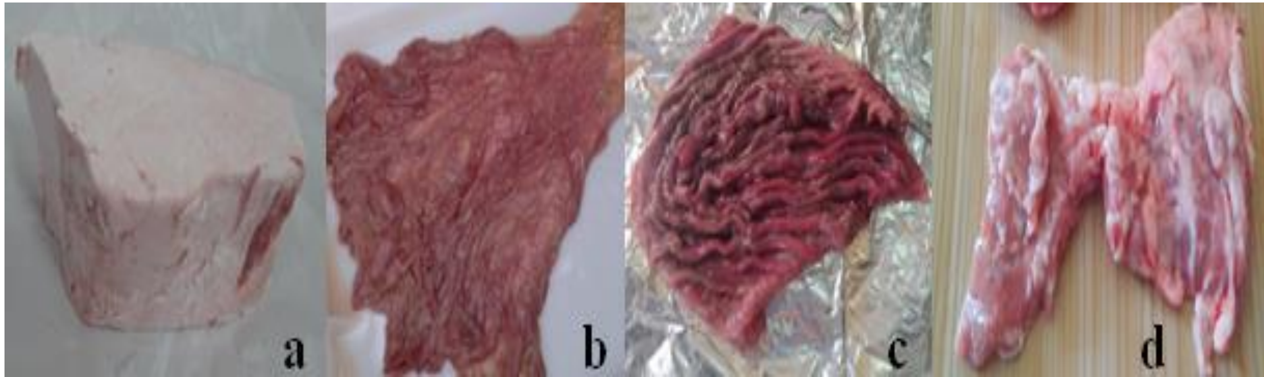


**Fig. 1.** Different samples used (a): fat, (b): rumen, (c): abomasum, (d): pancreas.

### Physico-chemical analyzes

The physicochemical characterization of the rumen, the pancreas, and the abomasum is carried out by determining the following parameters: the protein assay (Lowry *et al.*, 1951), the total lipids assay (Folche, 1957), the pH measurement, the determination of dry matter, ashes content and determination of humidity (Baleh, 2018).

The determination of the lipid parameters in the fat of the bump is carried out by measuring Total cholesterol, and triglycerides. HDL and LDL were determined using a colorimetric method by an autoanalyzer and using the reagent kit and the form specific to each parameter (Meiattini *et al.*, 1978).

### Lipase extraction protocol and confirmation of the presence of lipase by infrared IR

The extraction of camel lipase was carried out according to the protocol of (Shahani *et al.*, 1975). 5 grams of each sample were placed in iced sucrose (0.01M / 15ml) at 4 ° C. The samples are homogenized using a mortar and pestle, in cold sucrose for 90s. The homogenate obtained is then centrifuged in conical tubes at 6000 g for 30 min by a centrifuge.

After centrifugation, the supernatant is subjected to precipitation with 50% (v/v) saturated ammonium sulfate (15 ml) with moderate stirring, left to stand for 30 min at 4 °C, and then centrifuged at 6000 g for 40 min. The resulting pellet was then dissolved in a sucrose solution (0.01M) and again saturated with 50% ammonium sulfate. The mixture is again centrifuged at 6000g for 40 min and the resulting pellet (0.5g) is dissolved in phosphate buffer (2.5ml) and used as an enzyme source (Zaidi, 2014).

Infrared spectrometry is mainly used for the qualitative analysis of a molecule. Our goal in using this method is to confirm the presence of lipase in the various samples prepared.

Infrared (IR) spectroscopy relies on the absorption of light by most molecules in the infrared region of the electromagnetic spectrum and converting this absorption into molecular vibration. This absorption corresponds specifically to the bonds present in the molecule. With a spectrometer, this absorption of infrared radiation by the sample material is measured as a function of wavelength (in the form of wavenumbers, typically 4000 to 600 cm-1).

The result is a spectrum that gives a distinctive "chemical fingerprint" that can be used to visualize and identify organic and inorganic samples (Keirsse, 2003).

### Determination of enzymatic activity

Numerous methods intended to measure lipase activity have been described in the literature. Most are based either on the disappearance of triglycerides or on the production of fatty acids (FA). In the present study, lipase activity was measured, using a colorimetric method, and the previously obtained enzyme solution was used for the assay of lipolytic activity. By incubating an emulsion containing 8 ml of olive oil, 0.4 ml of phosphate buffer, and 1 ml of the solution containing the lipase (lipase extract), for one hour with stirring. The enzymatic reaction in the emulsion system was stopped by adding 1.5 ml of a 95% (v/v) acetone and ethanol solution. The lipase activity was determined by the spectrophotometer, the absorbance was measured at 715 nm, using olive oil as a substrate and linoleic acid as a standard. The activity is determined in enzymatic unit: the enzymatic unit is the amount of enzyme that catalyzes the transformation of a certain amount of substrate per unit of time (Zaidi 2014).

The concentrations of the lipase in the extract are calculated according to the following formula***:*** ***A0 (IU / L / min) = Ci - Cf / 60min***

With: ***A0***: enzymatic activity in international unit/ L/min; ***Ci***: Initial concentration of linoleic acid in (mol/L); ***Cf***: Final concentration of linoleic acid continued in one liter of olive oil in (mol/L); ***60min***: the incubation time of the lipase extract with the substrate (olive oil emulsion).

The measurement of the enzymatic activity in one gram of the tissue was carried out using the following formula: ***A1 (IU / g) = A0 x 10 / B***

With : ***A1***: the enzymatic activity of lipase in one gram of the tissue in international units per gram; ***A0***: enzymatic activity in international unit/L/min; ***10***: dilution factor; ***B***: is equal to 0.2 x 103 g.


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## RESULTS

### Phsico-chemical analyzes

The results of the physico-chemical analyses of the abomasum, pancreas, and rumen and the results of the biochemical analyses of the fat are shown in Table 1 and Table 2.

### Analysis of different lipase extracts by infrared IR spectroscopy

This method allowed us to confirm the presence of lipase in the extracts at two levels, one qualitative (indicates the existence or absence of the component sought) and the other quantitative (expresses the amount of the compound in the sample).

Figure 2, shows the spectrum that we have considered as a reference spectrum. Furthermore. Figure 3, shows the spectra of the extracts obtained by infrared IR spectroscopy. According to Andrade *et al.* (2016), the characteristic bands of lipase are found in the spectral region from 1100 cm-1 to 1700 $cm^{-1}$.

**Table 1.**

Physico-chemical parameters of different organs studied

| Parameters | Pancreas | Rumen | Abomasum |
|---|---|---|---|
| Proteins (%) | 21.48 ± 0.03 | 22.94 ± 0.05 | 20.18 ± 0.02 |
| Total lipids (%) | 5.15 ± 0.01 | 4.91 ± 0.01 | 2.15 ± 0.03 |
| pH | 6.4 ± 0.01 | 5.7 ± 0.07 | 4.3 ± 0.06 |
| Dry matter (%) | 35.14 ± 0.05 | 27.91 ± 0.03 | 26.78 ± 0.08 |
| Ashes (%) | 2.14 ± 0.01 | 3.16 ± 0.01 | 1.95 ± 0.02 |
| Humidity (%) | 64.86 ± 0.10 | 72.09 ± 0.50 | 73.22 ± 0.14 |

**Table 2.**

Biochemical parameters of dromedary fat

| Parameters | Dromedary fat |
|---|---|
| Fat content (%) | 75.04 ± 0.50 |
| Cholesterol (mg/dL) | 125.48 ± 1.26 |
| Triglycerides (mg/dL) | 30.45 ± 1.02 |
| HDL (mg/dL) | 48.94 ± 1.35 |
| LDL (mg/dL) | 110.24 ± 1.45 |

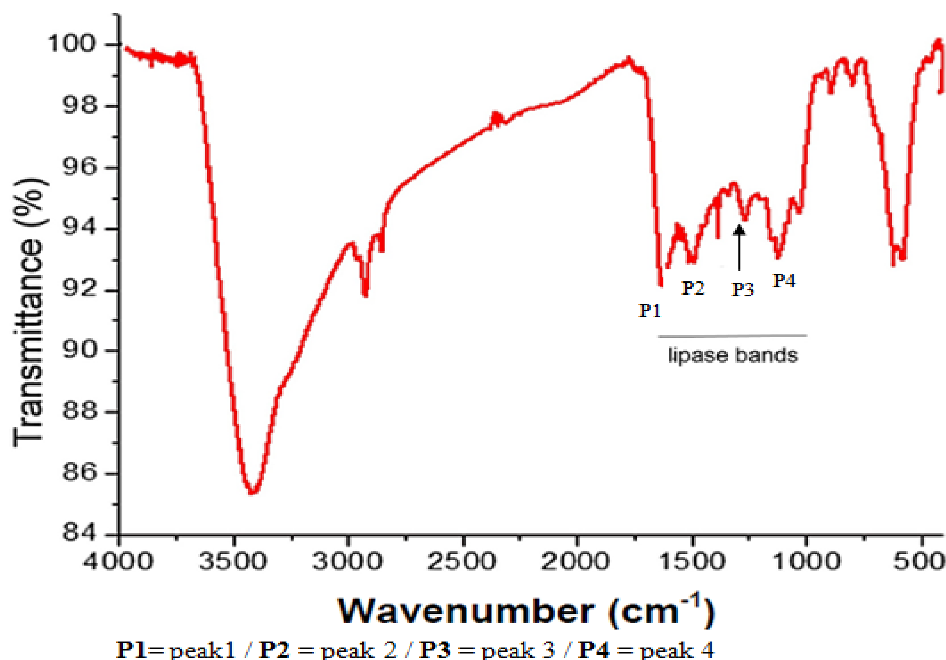


**Fig. 2.** Spectrum shows the characteristic peaks of lipase.


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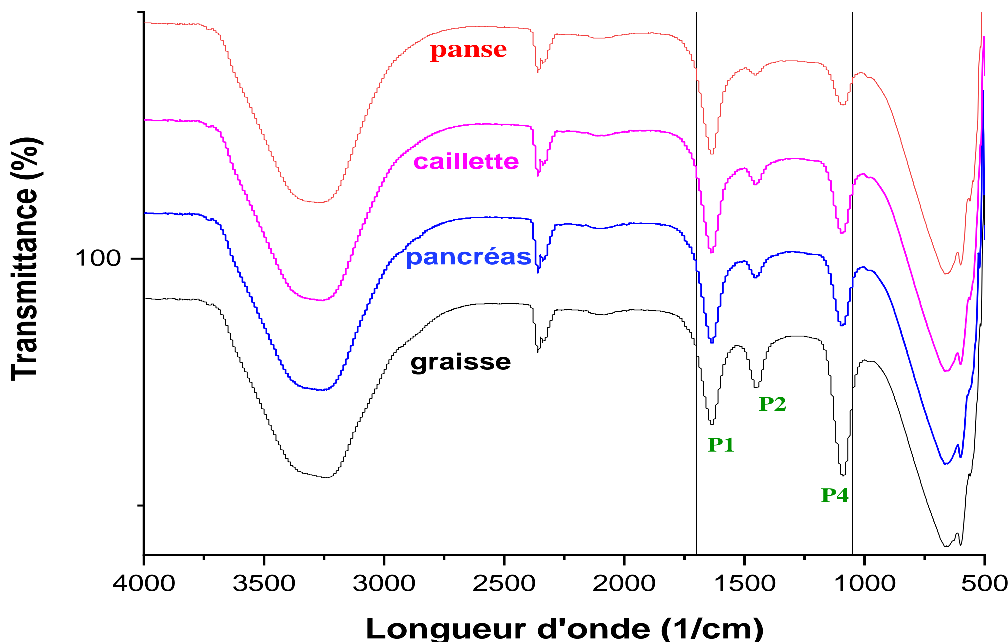


**Fig. 3.** Spectra of different samples: P1 = peak 1; P2 = peak; P4 = peak 4.

The depth of the spectrum peaks refers to the amount of the component in the sample where the low value of "Y" indicates that the sample has the highest amount of the component. Table 3, shows the Y and X values for each sample. Note that all the values of the X are equal to the level of the samples in the same peak (1st peak / X = 1640, 2nd peak / X = 1460, 4th peak / x = 1100) on the other hand the values of the Y are different. In addition, it's noted that the highest values of Y were observed in the rumen followed by the pancreas and the abomasum, and at the end, we find the fat which has low values compared to the other samples, which explains why the depth of the peaks.

The use of infrared allows us to identify and quantitatively assess the presence of a chemical compound, so from these results, we see that fat is the organ that contains the most amount of lipase.

**Table 3.**

**Y** and X values for different organs analyzed

| Organs | 1st peak / X=1640 | 2nd peak / X=1460 | 4th peak / x= 1100 |
|---|---|---|---|
| Abomasum | Y =72.9 | Y= 86.7 | Y= 65.2 |
| Fat | Y= 73 | Y= 81.1 | Y= 63.4 |
| rumen | Y= 72.7 | Y= 88.6 | Y = 82.6 |
| Pancreas | Y= 72.8 | Y= 86.6 | Y = 77 |

**Analyzes the spectra of the effect of different lipase extracts on olive oil**

The spectrum was characterized by the breaking of ester bonds between fatty acids and glycerol. In addition, the band at 3006 $cm^{-1}$ was a process (hydrolysis) fingerprint associated with the CH stretch vibration of the Cis double bond. The position of the band remains almost unchanged, but it underwent very little displacement during the first hour of hydrolysis.

The IR range between 3380 and 3610 $cm^{-1}$, showed the presence of bands around 1744 and 1745 $cm^{-1}$, the first was attributed to the harmonic of the carbonyl ester absorption glycerides, the second is due to the presence of a large proportion of side products of the enzymatic reaction such as alcohols, aldehydes or ketones and in particular the drawing of OH groups.

It is well known that after heat treatment or lipolytic action, certain infrared bands, especially in olive oil, are subject to displacement. The formation of the OH group due to the lipase reaction was revealed by the appearance of a band at 3270 $cm^{-1}$. This is explained by the decrease in intensity of the C=O ester band and the acyl chain (Guillen *et al.* 2000). The different bands observed and their frequencies are summarized in Table 4 and Figure 4.


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**Table 4.**

Differentiation of different peaks

| Frequency ($cm^{-1}$) | Type of bonds |
|---|---|
| 3388 | ν O-H |
| 3007 | ν C-H |
| 2922, 2853 | ν $CH_2$, $CH_3$ |
| 1744 | ν C=O |
| 1461 | δ$CH_2$ |
| 1161 | ν C-O |
| 721 | δ$CH_2$ |

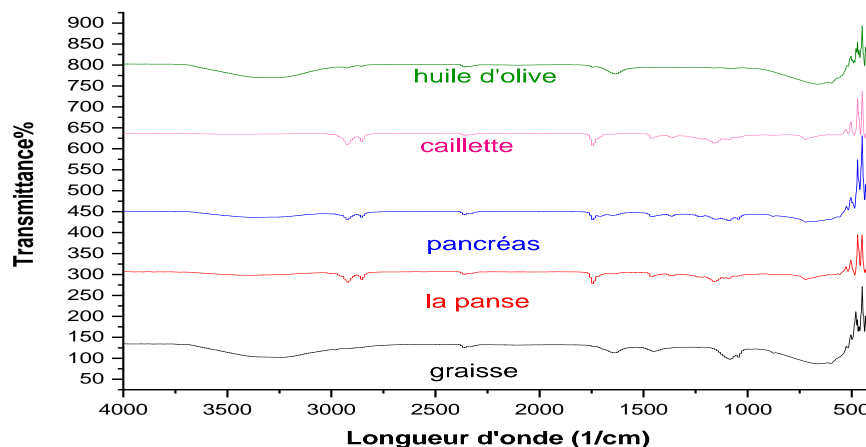


**Fig. 4.** Spectra of different lipase extracts with olive oil.

**Determination of the enzymatic activity of lipase in one gram of sample**

Lipase has many substrates that are used in the assay among these substrates: tributyrin, tween 80 olive oil ... etc. The specificity of the substrate is the ability of the lipase to catalyze the hydrolysis of a single type of substrate. The results obtained revealed that the use of olive oil would strongly induce the production of lipase (Rihani, 2012). This is why we chose virgin olive oil as a substrate in our experiment.

The values of the enzymatic activity are expressed in UI/L, the results obtained from Table 5, show that the highest activity of the lipase was observed in fat (46 IU/L) Follow-up by the rumen (30.17 IU / L), then the pancreas (27.18 IU/L) and the abomasum (26.122 IU/L).

**Table 5.**

Lipasic activity values in UI/L extract, in UI/g of the pellet, and UI/g of the tissue

| Samples | Extract activity (IU/L) | Pellet activity (IU/g) | Tissue activity (IU/g) |
|---|---|---|---|
| Pancreas | 27.18 | 1.359 | 0.1359 |
| Abomasum | 26.122 | 1.306 | 0.1306 |
| Fat | 46.128 | 2.306 | 0.2306 |
| Rumen | 30.17 | 1.508 | 0.1508 |

## DISCUSSION

The protein contents of the samples analyzed show no significant difference between the three organs studied. The contents obtained for the latter three are comparable. These rates appear higher than those reported by Kilgour (1986) (19.6%) and Kamoun (1995) (18.7%). In addition, some authors advance identical figures: 20.5% according to Dawood *et al.* (1995) and (22.7%) according to Kadim *et al.*, (2006), these results are confirmed by Ould Belkhir (2013) who found values (20 to 23%).

The protein content of camel meat varies from 20.99% to 22.98%, on the other hand in males varies from 21.58% to 22.80%. Therefore, our results agree and do not exceed the standards. According to Baleh (2018), we can explain these variations by the physical effort of the animal and the diet and age.

Regarding the results relating to total lipids, our results agree with those obtained by Kadim et al., (2006), (4.4%) and Alkanhal (1995) (4.1-10.6%) and higher compared to those reported by Ould El Hadj et al., (2002) who found values ranging from 1 to 2%. Bouras and Moussaoui (1995), confirm that the lipid content varies according to the type of muscle and the age of the animal. Yagil (1982) studied the effects of age, sex, and location on the composition of camel meat. They have shown that the fat content increases with age.

The results relating to the determination of pH, dry matter, ashes content, and humidity show fairly similar figures for the three organs and similar to the results obtained by Baleh (2018), Ould El Hadj et al., (2002), Daabouz et al., (2010) and Kadim et al,. (2006) for camel meat.


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The fat content (75.04%) of our sample is lower than that of Guessoum et al., (2019) who found values of 84.87%. The variations in body lipids are very poorly understood in dromedaries, but they are variable according to physical activity and according to the nutritional and physiological state (age, sex, castration), the size of the hump increases, and the percentage of body water decreases during the rainy season, when the animal reconstitutes its lipid reserves (Nasser *et al*., 2015).

The levels of the biochemical parameters Cholesterol, triglycerides, HDL, and LDL of our sample are lower than those of Benmoussa *et al.* (2020) who reported results of CH: 128.92 mg/dL, TG: 33.15 mg/dL, HDL: 50.34 mg/dL, LDL: 114.91 mg/dL.

Camel fat was used to relieve hemorrhoidal pain and hump fat was used to remove tapeworm (Taouti, 2017). By, its fat the dromedary provides appreciable food resources, triglyceride hydrolysis produces the release of two fatty acids and a monoglyceride which rapidly enters muscle, myocardial, and fat cells (Benyattou, 2018).

Cholesterol levels are affected by various factors, for example, composition of the food intake, age, sex, breed, season, gestation, lactation, and liver and bile duct disease. The increased concentration of cholesterol may be due to insulin, which plays a direct role in the metabolism of adipose tissue (Boudebza, 2015).

The olive oil spectrum was considered as a benchmark for comparing the different spectra. We observe the appearance of a few bands and the disappearance of the others, the peak in the spectral region of 500 $cm^{-1}$ to 800 $cm^{-1}$ is shared in olive oil, pancreas, and fat. At the region of 1000 $cm^{-1}$ to 2500 $cm^{-1}$, there are common peaks between the spectra of the extracts with olive oil, while the spectrum of olive oil contained only two peaks. Among these common peaks.

At the region of 2700 $cm^{-1}$ to 3700 $cm^{-1}$ the olive oil spectrum shares the same peaks with the other spectra in two different spectral zones, one from 2700 $cm^{-1}$ to 2850 $cm^{-1}$ which contains two peaks common between the spectrum of olive oil and the spectra of the rumen, pancreas, and abomasum, the other peak of wavelength 3250 $cm^{-1}$ common between the spectrum of olive oil and the fat and pancreatic spectra so the difference between all these peaks is in depth.

The appearance of peaks indicates the creation of new bonds, which indicates the formation of new products due to the catalytic effect of lipase from different organs on olive oil. The disappearance of the peaks demonstrates that there are disturbances of the bonds those which have been replaced by other new bonds of new products formed.

According to the analysis of the spectra, it can be seen that the effect of lipase from different organs on olive oil was different despite the fact that the same extraction protocol was applied in the same conditions of pH and temperature, and also we carried out the same catalytic reaction with the same substrate, therefore from this analysis and our previous information we can interpret these results by the specificity of structure of the lipase which differs from organ to organ, the location of the enzyme and its action at the different organ used and also by varying the favorable conditions that provide through these organs.

The values of the activity of the lipase of different samples in one gram of the pellet, it is observed that there is a considerable rate in the fat at 2.306 UI/g, followed by the rumen by a value of 1.508 UI/g then the pancreas and the abomasum that have similar values 1.359 UI/g, 1.306 UI/g respectively.

The results also show values of the activity of the lipase of different samples in a gram of the tissue, the fat occupied the highest value at 0.2306 UI/g, followed by the rumen at 0.1508 UI/g then the pancreas and the abomasum had Values close to 0.1359 UI/g, 0.1306 UI/g respectively. We find that the three pancreas, the abomasum, and the rumen have almost the same amount of lipase.

Besides, the fat has a larger amount of lipase by contribution to the three organs, this result has been confirmed by Boudiaf (2005) which confirms that the particular lipases located in the microsomes of adipocyte of the adipose tissue of the dromedary, This is due to its richness in lipid. Some parameters influence the hydrolysis efficiency of the lipase. These parameters are on the one hand the pH, the variations of the temperature, and on the other hand the emulsifiers, the type of oil, and the amount of the substrate. Our results are lower than those reported by Lagrari (2019) who studied lipase activity using 4 types of substrates olive oil, sunflower, glycerol and Tween 80, it found a low value of activity In the case of olive oil 2 UI/g by the values were much better at sunflower 3.5 UI/g, glycerol 4.9 UI/g, tween 80 3.9 UI/g.

According to Kouadio (2016), the type of olive oil causes a significant difference in lipase activity because they have an affinity for polyunsaturated AG-rich oils such as linoleic acid. These results are similar to those of Santos *et al*,. (2013) who have shown that the lipases of the fruit seeds of passion and sunflower have had a similar hydrolytic activity on the oils rich in linoleic and linolenic.

In our experience, ethanol was used to stop the enzymatic reaction; the latter is one of several factors that affect the activity of lipase. When ethanol is excessive can lead to inactivation or a decrease in biocatalyst activity by maintaining the active structure of lipases (Kouadio 2016). Abigor et al., (2002) showed that the maximum catalytic activity of lipases is obtained at a pH 7.5. Some lipases such as those studied by Enujiugha et al., (2004); Barros *et al.,* (2010) have an optimum pH equal to 7.

The pH acts on the enzymatic activity, on the properties of the interface in a multiphase system, on the solubility of the reagents in the medium as well as the sharing of the enzyme between the aqueous phase and the interface (Barros *et al.,* 2010). According to Moussavou *et al.* (2013), the temperature generally causes a drop in yield when the temperature of the reaction is above the physiological temperature of the


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activity of the lipase. Enzymes are denatured at high temperatures and lose their active conformations.

The variations of all these values can be explained according to two aspects, first of all, they depend on many specific factors of the dromedary, such as race, the age of the animal, its diet, its genetic type, its state of fattening its Sex, fashion of breeding and slaughter ... etc. Secondly, by the accuracy of the method used, and their polarity, the samples were not fresh and should have been used immediately after slaughter, the lack of necessary equipment (cold centrifuge x 15000g and polytron) which we did not allow to follow the protocol and replace them with other available equipment (mixer / 6000g centrifuge), the nature of the solvents used, the conditions of the medium and mainly, the region and the harvest period of the part used for extraction.

## CONCLUSION

The development of new biotechnologies and bio-industries is a major challenge for the future. This development requires the development of biocatalysts such as lipases which remain among the most important biocatalysts to carry out reactions in aqueous and non-aqueous media. The study of the physico-chemical characteristics of the pancreas, abomasum and rumen (respectively) shows a very large rate of protein (21.48%, 20.18%, 22.94%), lipid (5.15%, 2.15%, 4.91%), ashes (2.14%, 1.95%, 3.16%), moisture (64.86%, 73.22%, 72.09%), dry matter (35.14%, 26.78%, 27.9%), tense that, biochemical analysis of fat show a considerable rate of cholesterols125.48 mg/dl, triglycerides 30.45 mg/dl, HDL 48.94 mg/dl and LDL 110.24 mg/dl. The results obtained from the dosage of the lipase revealed that the highest enzymatic activity observed in fat (0.2306ui / g), compared to the other organs, pancreas 0.1359 UI/g, abomasum 0.1306 UI/g, the rumen 0.1508 UI/g. These results were also confirmed by the infra-red.

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## AUTHOR CONTRIBUTIONS

Ammar Touhami LAICHE and Chaima BENINE formulated the problem and the work objective and contributed to the analysis of the results and the writing of the article.

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## CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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