

Research Article



Effects of Treatment by Refined Soybean and Extra Virgin Olive Oils on Lipid Profile and Lipasic Activity in Wistar Albinos Rats

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ABSTRACT

The aim of this study was evaluating the effects of two oils consumed in Algeria, one from the extra-virgin olive, *Rougette* variety, rich in MUFA and the other one from the refined Soybean, rich in PUFA, on lipid profile and lipasic activity in *Wistar albinos* rats. The olive oil was ranked among "Extra-virgin olive oils (EVOO)" according to its physicochemical characteristics. Fatty acids assessment has shown a wealth of the olive oil in MUFA (80.12%), the oleic acid (C18:1, ω -9) is the predominant fatty acid (76.69%). Soybean oil, rich in PUFA (60.35%), represented by linoleic acid (C18:2, ω -6 at 53.67%). Olive oil has represented a high content in α -tocopherols (340.32mg/kg) compared to refined soybean oil (130.98mg/kg). The biological study showed that olive oil taken discontinuously for 45 days, resulted in an increase of the lipase activity and HDL-c levels (anti-atherogenic) and a decrease in LDL-c levels (atherogenic) compared to that administrated daily. Continuous administration of soybean oil for 25 days has improved the lipid profile resulting in a very highly significant increase of good cholesterol (HDL-c) and no effect on bad cholesterol (LDL-c); this treatment stimulated serum lipase activity for both administration periods. High lipase activity was also observed after a treatment with both oils taken alternately, however, no significant effect on HDL-c and LDL-c was noted after the two treatment periods.

Keywords: Olive oil, soybean oil, monounsaturated fatty acid, polyunsaturated fatty acid, lipid profile, lipase activity.

INTRODUCTION

Currently, the fatty acid composition of edible oils was one of the main criteria in their nutritional value determination. Several studies have shown a correlation between major edible fatty acids and their health benefits. Replacement of food grade saturated fatty acids (SFA) by monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) remains the best nutritional strategy in preventing cardiovascular diseases.¹

Short-chain saturated fatty acids, of C:10 - C:16, especially palmitic acid (C:16) are highly atherogenic by involving an increase in total cholesterol and LDL-c fraction, and a decrease of HDL-c.² MUFA such as C18:1 (ω -9), have a hypocholesterolemic action by decreasing LDL cholesterol while HDL cholesterol is maintained or increased.^{3,4} n-6 and n-3 PUFA (C18:2, ω -6 and C18:3, ω -3) have also a lowering effect on cholesterolemia, especially on LDL-c level.⁴

Olive oil consumption, oil containing a large quantity in MUFA (the major fatty acid is C18: 1, ω 9) and antioxidant compounds such as: polyphenols and tocopherols may improve the lipid profile in plasma; which could contribute on the prevention of cardiovascular diseases.⁵⁻⁷

Consumption of refined soybean oil, containing about 61% of PUFA and 24% of MUFA, could reduce total cholesterol and LDL cholesterol levels. Similarly, its high

content in alpha-linolenic acid provides it anti-thrombotic and anti-arrhythmic properties.⁸

Since there is no oil in nature rich in SFA, MUFA and n-6, n-3 PUFA; for a benefic effect on health, it will be interesting to limit SFA intake and increase the intake of MUFA and PUFA, with a PUFA ratio n-6/n-3 close to 5.⁹ Could olive and soybean oils combined use fulfill this role and improve lipemia?

In this context, the aim of the present study is to compare the effects of both oils consumed in Algeria, one from extra-virgin olive produced in Algeria and the other one from soybean imported and refined in our country, on lipid profile and lipase activity of normal *Wistar albinos* rats.

These oils being very used in the diet of Algerian population, we tried to determine if their consumption could harm health of Algerian and expose them to cardiovascular diseases.

MATERIALS AND METHODS

Plant material

Olives used in this experiment have been regularly collected from olive trees of *Rougette* variety (over about 4 years old), in the East of Algeria, specifically beside a private orchard called "*Djnen El Arbi*" in Guelma. Sampling was manually realized during the Olive oil Campaign 2011/2012 in mid-November. Soybean oil (*el'éo*) comes from SPA CEVITAL manufactory, Béjaïa, Algeria.



Fat extraction

From olive oil

The extraction was conducted in an oil mill by a continuous process according to the following steps: washing, grinding, kneading and centrifuging (Italian process *Alfa Laval*). Samples of oil were collected from the first pressure then conserved in dark sterilized bottles in the freezer at 4 °C until their use.

From soybean oil

It was extracted by classic refining following the next steps: degumming, neutralization, discolouration, and deodorization.

Biochemical analysis of the three fat materials used in this study

Fatty acid assay by GC

Assay of fatty acids methyl esters (FAMES) was carried out by gas chromatography (chromatograph: Agilent 6890N Network GC, FID detector and SPLIT injector, equipped with a DB23 Agilent 122-2362 capillary column (60m x 0.25mm x 0.25 μ m), the carrier gas is hydrogen H₂, provided at pressure of 14,84psi with a constant flow of 1ml/min.

The maximum temperature of the column is maintained at 260°C, the maximum oven temperature was 325°C, the maximum temperature of the injection was 325°C and that of detector was 250°C. The injected volume was 1 μ l. The fatty acids were identified by comparison of retention times with those of standard fatty acids.

Determination of minor compound

Chlorophyll and carotenoids

Chlorophyll and carotenoids contents were determined according to the method described by Minguez.¹⁰ The results were expressed in ppm (mg/kg) of oil. Three trials were performed.

α -Tocopherols

α -Tocopherols content was determined by HPLC (WATERS 1525 with binary pump) over an analytic column in normal phase WATERS SPHERISORB (3 μ m of Silica / 150mm x 4.6mm i.d) according to the international standard : ISO 9963.¹¹ 2g of extracted oil was dissolved into 25ml of hexane and filtrated. 20 μ l from the solution was manually injected. α -Tocopherols separation was realized using an isocratic elution hexane/2-isopropanol (95.5:0.5 v/v) at a flow rate of 0.8ml/min. Visible UV detector WATER 2487 was set on the wavelength of λ =292nm. External calibration curve was prepared to calculate α -tocopherols quantity present in the sample (r^2 =0.999). Samples were injected in triplicate. Results were expressed in mg of α -Tocopherol/kg of oil.

Biological experiment

Animals and diet

72 *Wistar albinos* male rats obtained from Pasteur institute of *Kouba*, Algiers were used in this experiment. After few days of acclimatisation in ambient temperature and a 24-hour day/night cycle where animals have been fed with a complete and equilibrated standard diet provided by *UAB*: National Unit of Animal Feed (Béjaïa). All animals have access to water and diet "*ad-libitum*". This study was performed according to the guidelines for animal care and use.¹²

Each oil was administrated by a single oral dose to animals: daily for some groups, discontinuously (1 day out of 2) for other groups and both oils by alternation (1 day out of 2 for each oil) during two periods; 25 and 45 days. The rats were well-maintained; weighing of animals was done every 5 days during the two treatment periods.

Trial protocol

After the acclimatisation, the rats were randomly divided into 12 groups (n=6) according to the trial protocol shown on the following table (1).

Table 1: Experimental Protocol

Periods	Groups (n=6)	Characteristics
P1 (25 days)	C1	Control group received daily water in the same conditions as other groups.
	OC1	Group treated daily by 0.9g of extra virgin olive oil /kg of body weight (bw).
	OD1	Group treated discontinuously by 0.9g of extra virgin olive oil /kg bw.
	SC1	Group treated daily by 0.9g of soybean oil /kg bw.
	SD1	Group treated discontinuously by 0.9g of soybean oil /kg bw.
	OS1	Group treated alternately by both oils.
P2 (45 days)	C2	Control group received daily water in the same conditions as other groups.
	OC2	Group treated daily by 0.9g of extra virgin olive oil /kg of bw.
	OD2	Group treated discontinuously treated by 0.9g of extra virgin olive oil/kg bw.
	SC2	Group treated daily by 0.9g of soybean oil /kg bw.
	SD2	Group treated discontinuously by 0.9g of soybean oil /kg bw.
	OS2	Group treated alternately by both oils.

All groups were fed with the same standard diet.



Table 2: Biochemical composition of fatty materials

Parameters	Content %				
	C	HS	Codex [1999]	HOEV	COI [2015]
C12 :0	1.60 ± 0.05	/	ND-0.1	/	ND
C14 :0	1.84 ± 0.14	/	ND-0.2	/	≤ 0.03
C16 :0	42.46 ± 0.34	10.89±0.02	8 - 13.5	11.21 ± 0.26	7.5 – 20
C18 :0	2.29 ± 0.10	3.90 ± 0.01	2.0 - 5.4	2.19 ± 0.01	0.5 - 5
C20 :0	/	0.00	0.1- 0.6	0.36 ± 0.01	≤ 0.6
C16 :1 (ω-9)	0.60 ± 0.06	/	ND-0.2	1.10 ± 0.01	0.3 - 3.5
C18 :1 (ω-9)	11.94 ± 0.01	23.38 ± 0.14	17- 30	76.67 ± 0.29	55 - 83
C18 :1 (ω-7)	/	1.48 ± 0.01		2.35 ± 0.05	
C18 :2 (ω-6)	37.04 ± 0.06	53.67 ± 0.21	48 – 59	5.45 ± 0.03	2.5 - 21
C18 :3 (ω-3)	2.23 ± 0.03	6.68 ± 0.05	4.5 – 11	0.68 ± 0.01	≤ 1
SFA	48.19	14.79		13.76	
MUFA	12.54	24.86		80.12	
PUFA	39.27	60.35		6.13	
Chlorophylls (ppm)	ND	ND		4.71 ± 0.01	
Carotenoids (ppm)	ND	ND		2.26 ± 0.06	
α-tocopherols (mg/kg)	225 ± 0.90	130.98 ± 5		340.32 ± 19	

C: Control fat, HS: soybean oil, HOEV: extra virgin olive oil.

Sacrifice and blood sampling

The sacrifice was realized at the end of each treatment period; blood was collected by decapitation in dry tubes and then centrifuged at 3500rpm / 15min, the serum obtained will be used for assay of blood lipid parameters.

Effects of oils on the lipid profile

Lipid profile (total lipids, Triglycerides, Total Cholesterol, HDL-c, LDL-c) was determined by colorimetric methods by a SECOMAM automatic analyzer using commercial kits Spinreact.

Effects of oils on serum LPL activity

Lipase activity was determined by colorimetric methods using a SECOMAM automatic analyzer using a commercial kit Quimica clinica.

Statistical analysis

The one-way analysis of variance (ANOVA) was used for statistical comparisons followed by *Dunnnett's* test. The results expressed as mean ± standard error with a significance level $p \leq 0.05$.

RESULTS AND DISCUSSION

Biochemical composition of the tree fat materials used in this study

Results of the biochemical analysis of oils are shown in Table 2. The fat matter of control diet contains a high

content of saturated fatty acids SFA (48.19%) represented by palmitic acid as the predominant fatty acid (42.46%).

Olive oil of local variety "*Rougette*" is characterized by a high content of monounsaturated fatty acids (MUFA) with a rate of 80.12%, represented by oleic acid as major fatty acid from the Omega-9 family (76.67%), 13.76% of saturated fatty acids (SFA) and 6.13% of polyunsaturated fatty acids (PUFA) represented by a dominance of omega-6 linoleic acid and traces of α-linolenic acid omega-3. This composition complies with COI standards¹³ and shows similarity with that of the olive oil of Algerian variety "*Grosse de Hamma*".¹⁴

For Soybean oil, the fatty acid levels comply with standards recommended by the Codex Alimentarius¹⁵ except for arachidonic acid (C 20:4 ω6) showing a lower rate than those standards. This oil, in contrast to the olive one, is characterized by a high content of polyunsaturated fatty acids represented by linoleic acid ω-6 (53.67%) and the ω-3 linolenic acid (6.68%).

Chlorophylls and carotenoids contents are 4.71 and 2.26mg/kg, respectively (Table 2), these rates are in agreement with those reported by Salvador¹⁶ and Ounni¹⁷ for both varieties "Oueslati and Chetoui".

The α-tocopherols, representing 90% of total tocopherols in olive oil,¹⁸ are the most common used form in vitamin E dietary supplements.¹⁹ The results show high levels of α-tocopherols in olive oil (340.32mg/kg) compared to

soybean oil and control fat (130.98mg/kg and 225mg/kg of α -tocopherols, respectively).

Effects oils on the lipid profile

Total lipids (TL)

After 25 days of administration of both oils, the results show that only OC1 and OD1 groups (treated with extra virgin olive oil daily and discontinuously, respectively) and SC1 (treated daily by soybean oil) have a highly significant increase in TL levels compared to the control. This result is related to high levels of total cholesterol (Figure 3) for the group treated by olive oil and high levels of triglycerids for that treated by soybean oil (Figure 2). SD1 and OS1 groups show similar results as control (Figure 1).

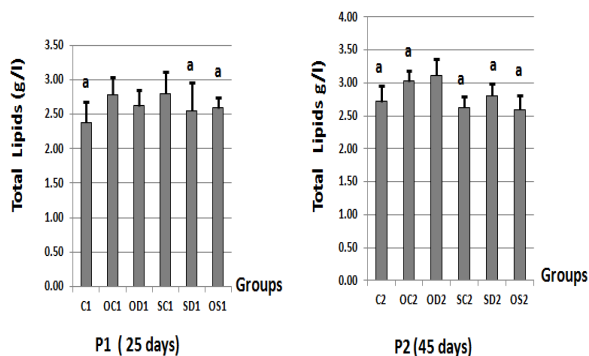


Figure 1: Effect of extra virgin olive oil and soybean oil on total lipids after the two Treatment periods. Values that don't show a letter have highly significant differences compared to the control ($p \leq 0.01$, P1 "25 days"; P2 "45 days")

After 45 days, we noted high levels of TL in OD2 group (treated discontinuously with extra virgin olive oil) with a very highly significant difference compared to control. This result is linked to high triglyceride levels, which is in agreement with the results reported by Baba;²⁰ while SC2 and OS2 groups showed a non-significant reduction in TL levels compared to control; this low hypolipidemic effect is probably related to the presence of fatty acid C18: 3 (ω -3) in small amounts in soybean oil.²¹

Triglycerids (TG)

The results obtained for triglycerids levels are mentioned in Figure 2.

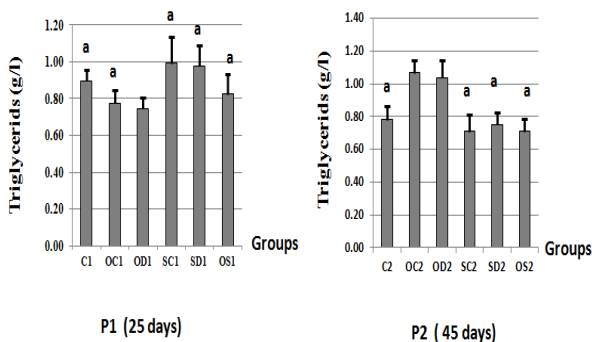


Figure 2: Effect of extra virgin olive oil and soybean oil on triglycerids levels after both treatment periods. Values that

don't show a letter have highly significant differences compared to the control ($p \leq 0.01$, P1 "25 days"; P2 "45 days").

After 25 days of administration of the two oils, we registered low triglycerids levels in OC1, OD1 and OS1 groups. Only the OD1 animals that represented a very highly significant difference compared to controls. This result shows that treatment with the extra virgin olive oil taken discontinuously result in a triglycerid-lowering effect, which is consistent with results of clinical studies of Covas²² and Vincent Baudry.²³

After 45 days of treatment, we noted in OC2 and OD2 groups high levels of TG compared to control with a very highly significant difference; showing that olive oil, administered daily or discontinuously, had a hypertriglyceridemic effect.

This effect is probably due to richness of this oil in oleic acid MUFA (C18: 1),²⁴ result in agreement with that of Baba.²⁰ While, these levels are slightly lower in SC2, SD2 and OS2 groups with no significant differences compared to the control.

These results are contradictory to those of Ide²⁵ and Vijaimohan²¹ showing that soybean oil, rich in PUFA, had a reducing effect on plasmatic triglycerids levels.

Total Cholesterol (TC)

Figure 3 shows the results of total cholesterol level levels assessment after both experimental periods.

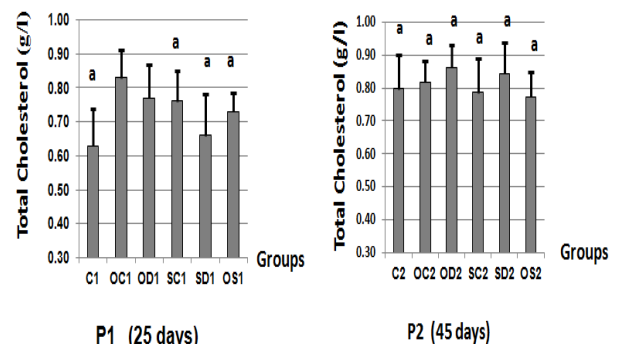


Figure 3: Effect of the extra virgin olive oil and soybean oil on total cholesterol levels after the two treatment periods. Values that don't show a letter have highly significant differences compared to the control value ($p \leq 0.01$, P1 "25 days"), the values designated by the same letter show no significant difference ($p > 0.05$, P2 "45 Days").

After 25 days of treatment with the two oils, we found that only OC1 and OD1 groups have resulted in an increase of TC levels in rats with a highly significant difference compared to the control.

These results suggest that extra virgin olive oil had a hypercholesterolemic effect after 25 days of treatment, which are in contradiction with results reported by Salsa-Salvador.²⁶

After 45 days of treatment, we registered that TC level in OC2 and OD2 groups are similar to the control's one,

suggesting that olive oil had no effect on serum TC; result in agreement with those found by Kontogianni.²⁷

While soybean oil administered daily and the two oils taken alternatively had a slight cholesterol-lowering effect probably related to the presence of linoleic acid^{28,29} and linolenic acid.

According to some authors,^{21,30} the presence of this fatty acid in diet has involved a cholesterol-lowering activity.

HDL-c

In Figure 4 are shown the results of HDL-c determination

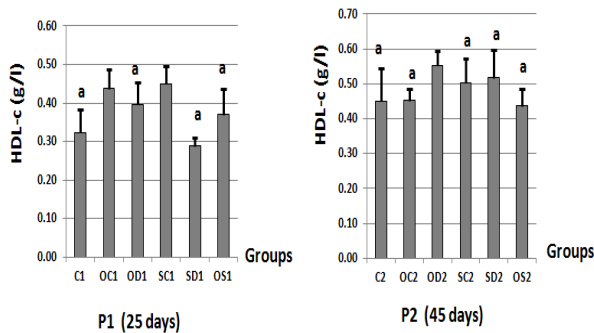


Figure 4: Effect of the extra virgin olive oil and soybean oil on HDL-c levels after two treatment periods. Values that don't show a letter have a highly significant differences compared to the control value ($p \leq 0.001$ in P1 "25 Days"); Values who do not share letter represents a significant difference ($p \leq 0.05$ in P2 "45 Days").

After 25 days of treatment with the two oils, we noticed that only OC1 and SC1 groups treated daily by olive oil and soybean have exhibited an increase in HDL-c levels with a very highly significant difference compared to control; this increase is probably due to the presence of oleic and linoleic acid in both oils.³¹

After 45 days of treatment, only OD2 (group treated with olive oil discontinuously) showed a significant increase in HDL-c levels compared to control. These results are in agreement with those of clinical studies reported by Kratz³² and Covas.²²

LDL-c

The results shown in figure 5 represent the LDL-c levels of groups treated by the two oils after the two periods.

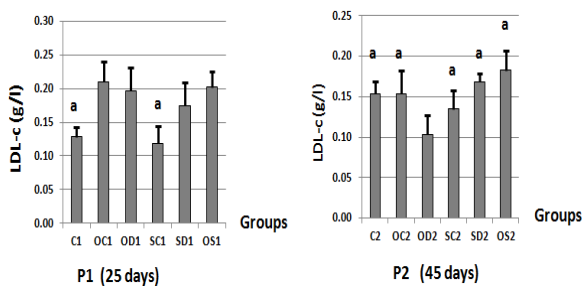


Figure 5: Effect of extra virgin olive oil and soybean oil on LDL-c levels after the two treatment periods. Values that don't show a letter have very highly significant

differences compared to the control ($p \leq 0,001$ in P1 "25 Days"; P2 "45 Days").

After 25 days of treatment, we found that LDL-c concentration in OC1, OD1, SD1 and OS1 are higher than that of control with very highly significant differences except the SC1 group, which is almost similar to the control.

After 45 days of treatment, we noted that only the OD2 rats had low LDL-c levels with very highly significant differences compared to control. Based on these results, we can deduce that the decrease in LDL-c levels caused by the discontinuous olive oil treatment is probably due to the presence of Omega-9 oleic acid, the major fatty acid in our olive oil⁴ and to phytosterols that are structural analogues of cholesterol in plants. They interfere with the intestinal absorption of cholesterol and can contribute to serum LDL-c lowering.³³

Effect of the two oils on serum LPL activity

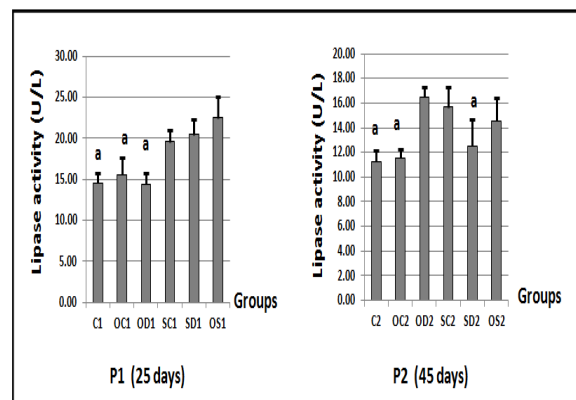


Figure 6: Effect of extra virgin olive oil and soybean oil on lipase activity after the two treatment periods. Values that don't show a letter have very highly significant differences compared to the control ($p \leq 0,001$ in P1 "25 Days"; P2 "45 Days").

From Figure 6, we noted that soybean oil administered to groups SC1, SD1 and OS1 for 25 days, resulted in a very highly significant increase of lipase activity compared to control group in contrast to OC 1 and OD1 ones (treated with extra virgin olive oil) in which results are similar to control rats. Based on these results, soybean oil would have exhibited a stimulating effect on serum LPL activity in rats; which could be related to its high content in PUFA (mainly linoleic acid ω -6).³⁴

After 45 days of consumption of both oils, we noticed an increase in serum LPL activity in all treated groups compared to control one, but only OD2, SC2 and OS2 groups that showed increased LPL activity with very highly significant differences. These results showed that the quality and quantity of the ingested oil has an impact on LPL activity; indeed, according to some studies, high lipid content in diet results in an increase in serum LPL activity in human and rat.³⁴⁻³⁶

CONCLUSION

The results obtained showed that olive oil administered to rats is of oleic type (ω -9 at 76.67%) and refined soybean oil is rich in linoleic acid (53.67%). Olive oil presented a high content of α tocopherols (340.32mg/kg) compared to the refined soybean oil (130.98 mg/kg).

Treatment with the two oils, monounsaturated and polyunsaturated showed different results depending on the degree of unsaturation and the length of treatment period.

After 25 days of treatment, olive oil administration daily and discontinuously and treatment by soybean oil daily at a rate of 0.9g/Kg bw increased serum TL. For triglycerids, only discontinuous treatment by olive oil exhibited a triglycerid-lowering effect. Regarding the total cholesterol, olive oil, taken daily and discontinuously, and even soybean oil administered continuously increased serum TC showing high levels of good cholesterol (HDL-c). For bad cholesterol, only soybean oil taken daily didn't exhibit an increase of this parameter. The measurement of the lipase activity showed that soybean oil and even the two oils taken alternatively increased this activity.

After 45 days of treatment, only discontinuous administration of olive oil increased serum TL and good cholesterol HDL-c and decreased LDL-c (bad cholesterol). Hypertriglyceridemia was observed in rats treated with olive oil daily and discontinuously. A high lipase activity, very highly significant, was recorded only in treated rats by olive oil taken discontinuously, the soybean oil treated daily and the two oils administered alternately.

From these results, it would seem that for a balanced lipid profile, good for health, the discontinuous intake of olive oil and daily administration of soybean oil for periods of 45 and 25 days respectively, would be preferred. From this study, we can conclude that the benefic effect of vegetable oils on health is related to the way of their use.

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