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SMEN AND ARGAN OIL AS A SOURCE OF BIOACTIVE NATURAL PRODUCTS: AN INSIGHT INTO THEIR FATTY ACID PROFILE, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES

Hanane BAKRIM[*1], *Ouiam EL GALIOU*[1], *Mostafa LAMHAMDI*[2,3], *Hamza EL MOUDDEN*[2], *Mounir HASSANI ZERROUK*[1], *Abdelhay ARAKRAK*[1], *Mohammed BAKKALI*[1], *Amin LAGLAOUI*[1]

Address(es):
[1] Biotechnology and Biomolecular Engineering Research Team, Department of Biology, Faculty of Science and Technology, Abdelmalek Essaâdi University, Tetouan, Morocco.
[2] Higher School of Technology of El Kelaa Des Sraghna, Cadi Ayyad University, El Kelaa Des Sraghna BP 104, Morocco.
[3] Laboratory of Microbial Biotechnologies, Agrosciences, and Environment (BioMAgE), Labeled Research Unit-CNRST N◦ 4, Faculty of Sciences Semlalia, University Cadi Ayyad, Marrakech, Morocco.

*Corresponding author: bakrimhanane@gmail.com

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ABSTRACT

Morocco holds a rich and diversified cultural heritage, conveyed by its artisanal products. These products include crafts and foods with biological benefits, including Smen and Argan oil. This study was carried out in view of evaluating the effectiveness of both artisanal products for potential application in various industries. The antioxidant and antimicrobial activities of these products were explored in this study. Free fatty acid content highlighted the abundance of Palmitic, Stearic, and Oleic acids. All samples reflected a substantial antioxidant power, with Artisanal Argan revealing the highest efficiency (IC_{50}= 1.5 mg/mL). *L. monocytogenes* biofilms were effectively suppressed by all samples (>90% except Artisanal argan showing >80% inhibition), Kinetic studies proved that the tested products could successfully inhibit *L. monocytogenes* and *S. aureus* growth, evidenced by the bacteriostatic effects of Smen samples from Tazrout and Had lgharbia regions. Commercial argan exerted the highest antifungal potential at both concentrations (>60%). Our findings suggest that both types of artisanal products are potent sources of natural preservatives and health-promoting agents, which highlights their potential as prospects for research in food science and medicine.

Keywords: Artisanal product; Antibacterial; Antioxidant; Argan oil; Smen; GC-FID

INTRODUCTION

Nowadays, there has been a noticeable trend among consumers in the demand for artisanal food products. This demand is mainly attributed to the notion that these products are essentially regarded as healthier, more genuine alternatives thanks to their production pathway, which primarily occurs in small-scale production facilities, therefore gaining wide popularity with consumers that continuously demand bio-based alternative solutions in the food industry **(Pasquali *et al.*, 2022)**. Morocco is one of the many countries that boast a wide and diverse culture, especially in the culinary sector, which represents an integral part of its cultural heritage, with a wide variety of characteristic dishes, spices, cooking fats and oils, and dairy products….All these culinary elements reflect and are influenced by the country's cultural, historical, and ethnobotanical diversity **(Ez zoubi *et al.*, 2022; Gagaoua and Boudechicha, 2018)**. The use and production of these artisanal products are practiced by a large section of the Moroccan population and are often considered the main occupation and an essential part of many communities, especially in rural or mountainous regions **(El Galiou *et al.*,2015).**

Argan oil is a type of oil obtained exclusively from Moroccan *Argania spinosa* trees. A plant endemic to Morocco, particularly in the southern regions. In 1998, the areas where argan trees are typically found (the argan forest) were designated as biosphere reserves by UNESCO **(Charrouf and Guillaume, 2018)**. Kernels from Argan nuts are broken, collected, and cold-pressed to obtain argan oil. According to the preparation process, two types of oils can be produced. Kernels can be subjected to breaking while raw to prepare cosmetic-grade argan oil, while roasted kernels are used to produce edible oil **(Mohammed *et al.*, 2021)**. For centuries, the argan tree has been an integral part of the local Berber population's livelihood and has been used as a staple of their economy. While the seeds were used for the preparation of argan oil, other parts of the plant (leaves, fruit pulp, wood…) were implicated in traditional medicine and have been used to treat several disorders, including fever, dermatosis, diabetes, and hypercholesterolemia **(Mechqoq *et al.*, 2021)**.

Both food-grade and cosmetic-grade argan oil have been reported to possess several medicinal properties, such as the treatment of juvenile acne, chickenpox, and the reduction of wrinkles, in addition to having anti-cardiovascular and anti-cancer properties **(Gharby and Charrouf, 2022; Guillaume *et al.*, 2019)**.

Smen, a type of fermented butter, is common in various cultures, the Moroccan version of Smen is a fermented salted butter containing 8 to 10% salt. It is traditionally prepared from fermented cow or goat's milk, and the resulting butter is washed continuously with salted water, Smen is then salted and stored under anaerobic and dark conditions for 3-6 months to promote maturation **(Benkirane *et al.*, 2022)**.

Smen offers a large chemical diversity that can present a certain level of variability, however, typical average values for the principal components are fat (81.34%), non-fat dry matter (4.96%), water (13.7%), salt (1.5%), and proteins (3.25%) **(Iradukunda *et al.*, 2018)**. Smen is also rich in microbial lactic flora, with the Bacillus genus being the dominant flora due to their capacity to metabolize FFAs **(Sarhir *et al.*, 2021)**. Smen is largely used as a food additive in Moroccan culinary practices due to its organoleptic properties; it provides a unique aroma capable of improving both the taste and flavor of the prepared dishes. This powerful aroma is induced by the high concentrations of ester compounds and carboxylic acids such as ethyl hexanoate, ethyl butanoate, butanoic acid, and hexanoic acid **(Sarhir *et al.*, 2021)**.

Smen has been transmitted from one generation to another for centuries. It has been traditionally used to treat skin diseases. This product is of particular interest since its preparation method is equally considered a way to help preserve butter and extend its shelf life since it allows a more stable and consistent formulation capable of withstanding long-term storage **(Kalam Saleena *et al.*, 2023)**.

Both products are integrated into Morocco's culinary heritage, and while their chemical composition has been extensively investigated, few studies have offered a comprehensive overview of their biological contribution to the dietary rituals of the Moroccan population. Therefore, this study was conducted to analyze the antioxidant and antimicrobial potential of Moroccan argan oil and Smen products. The significance of this study relies on the fact that it provides a comprehensive insight into the biological importance of the studied products. The results obtained will significantly contribute to our understanding of the biological activities of these natural products as well as identifying new potential alternatives to food preservatives.

MATERIALS & METHODS

Sample preparation

Argan oil

Two argan oil samples (*A. spinosa*) were selected for this study. Both samples were obtained from two different sources. Artisanal Argan (AA) was obtained from Argan trees grown in the Souss-Massa region, particularly from the Amaghouz village located in the Anzi district (Tiznit region). The oil was extracted following a traditional extraction method employed by Berber women. This process consists of five phases: the removal of the fruit, the crushing of the shell with two stones, the roasting of the almond, the grinding of the roasted almond with a stone grinding wheel, and finally the manual pressure of the dough to extract the oil, which is then decanted. The second sample, Commercial Argan (CA), is a commercial oil purchased at the local market in Tangier. Both samples were selected to compare their chemical characteristics and properties. The sample obtained by the traditional method represents an example of artisanal production, while the sample purchased at the local market is likely to be from a more standardized commercial production.

Smen samples

Three samples of ''Smen'' were obtained from different regions in Northern Morocco to encompass the local variations existing in the Moroccan market. The samples included two locally handmade products obtained from local vendors. Sample 1, Tazrout Smen (TS), was acquired from the Tazrout region (35°01'20.1"N 5°32'03.9"W) and sample 2, Had lgharbia Smen (HS), was obtained from Had lgharbia local market (35°31'20.4"N 5°55'43.8"W). The third sample, Commercial Smen (CS), is a commercially available product purchased from the local market in Tangier. All Smen samples were prepared following traditional practices consisting of placing traditional butter (locally made butter) in heating pots along with salt and water (one cup of water for every kilogram of butter). Once the butter melts completely and reaches its boiling point, flour is added to absorb any excess water (one tablespoon for every kilogram of butter), the obtained liquid is filtered and stored in sterilized glass jars until solidification. All samples were prepared and stored under similar conditions to ensure consistency and maximum product integrity.

Determination of physicochemical properties

Free fatty acid composition

The free fatty acid profile of the Argan and Smen samples was analyzed following the procedures outlined by the International Organization for Standardization (ISO) **(ISO 12966-2, 2017)**. To convert the fatty acids (FAs) into their corresponding methyl esters (FAMEs, fatty acid methyl esters), a solution was prepared by mixing 0.1 g of oil with 2 mL of hexane and 100 µL of 2N methanolic potassium hydroxide. The resulting solution was agitated. After trans-esterification, the FAMEs in the hexane layer were separated and then dehydrated using 1g of anhydrous sodium hydrogen sulfate. Gas chromatography, specifically utilizing a hydrogen flame ionization detector (FID) and a BPX70 capillary column (60 m × 0.32 mm i.d.; 0.25 µm film thickness), was employed for the FAMEs analysis. High-purity helium gas was used as the carrier gas, flowing constantly at a rate of 1mL per minute. A 1µL sample injection was performed with a sample split ratio of 1:50. Both the injection and detection ports were maintained at a temperature of 250°C. The temperature in the oven was initially set at 170°C for 3 minutes and then programmed to increase gradually at a rate of 4°C per minute until it reached 230°C, where it was held for 15 minutes. The fatty acids were identified by comparing their retention times to those of known FAMEs with established fatty acid compositions, and the results were reported as a percentage of the total fatty acids.

Determination of secondary metabolites

Analysis of the phenol content

A. spinosa oils, together with Smen samples were first tested for the determination of their total phenolic content according to the standardized method described by the International Organization for Standardization (ISO) **(ISO 14502-1: 2005, 2005) (Anesini Ferraro and Filip, 2008)**. Briefly. 1 mL of each sample (100 mg/mL for AOs and 10 mg/mL for SOs) was mixed with 5 mL of Folin-ciocalteu reagent [1:10]. After 8 minutes of incubation at room temperature, 4 mL of 7.5% sodium carbonate was added, and the solution and further incubated at room temperature for 1 hour.

The absorbance was measured at a wavelength of 765 nm. The quantification of total phenolics was determined using the regression equation based on a calibration curve established with gallic acid (10-50 µg/mL; r^2=0.986) under conditions identical to those of the sample. The results are expressed in milligrams of gallic acid equivalent per gram of sample (mg GAE/g Sample).

etermination of the total flavonoid content

The total flavonoid content (TFC) was investigated using the protocol adopted by **(Dehpour *et al.*, 2009)** with some modifications. The samples were diluted to yield a concentration of 1 mg/mL. 50µL of each sample was added to tubes containing 150 µL of ethanol and later supplemented with 10 µL of aluminum chloride and 10 µL of potassium acetate (1M). After 30 minutes of incubation at room temperature, the absorbance of the sample was measured at 415 nm. The content of flavonoids was deduced with the help of a calibration curve prepared with Rutin (0.0005-2 µg/mL; r^2=0.947). TFC was expressed as a milligram of Rutin equivalent per gram of sample (mg RE/g Sample).

Screening of the antioxidant potential

Sample preparation

Stock solutions of *A. spinosa* oils (100 mg/mL) were diluted in ethanol to yield the following concentrations: 100; 50; 25; 12.5; 6.25; and 3.125 mg/mL for each oil sample. Simultaneously, Smen samples were prepared by mixing 40 mg of each sample with 4 mL of ethanol, resulting in a final concentration of 10 mg/mL. A dilution series was prepared to yield 10; 5; 2.5; 1.25; 0.625 and 0.3125 mg/mL.

Free radical scavenging assay (DPPH assay)

A stock solution of DPPH was prepared at an initial concentration of 0.1 mM. Immediately before testing, a dilution of the stock solution was prepared to reach the final concentration of 0.3 mM required for the experiment.

On a 96-well microplate, each dilution was deposited (143 µL), and then supplemented with 57 µL of the DPPH solution. 143 µL mixture of extract at different concentrations and 57 µL of ethanol were applied as blanks, whilst the negative control was obtained by mixing 143 µL of ethanol with 57 µL of DPPH. The microplate was placed in the dark and at room temperature for 30 minutes. The samples were read using a spectrophotometer at 517 nm **(Bakrim *et al.*, 2021)**. The antioxidant activity (AA%) was calculated as a percentage of the discoloration of the DPPH solution in ethanol using the following formula:

$$\mathbf{AA\%} = \left[\frac{(\mathbf{Abs_{Control}} - \mathbf{Abs_{Sample}})}{\mathbf{Abs_{Control}}}\right] \mathbf{x100}$$

With:

AA%: Antioxidant Activity

Abs: Absorbance at 517 nm.

The ferric-reducing antioxidant power (FRAP assay)

The method described by **(Lfitat *et al.*, 2021)** (with modifications) allowed the determination of the reducing power of the extracts by measuring the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) by the present antioxidants. This reaction is accompanied by the production of a characteristic blue color. 2 mL of each sample is added to 1 mL of 0.2 M phosphate buffer and 1 mL of 1% potassium ferricyanide. After a 20 minutes water bath at 50°C, the reaction was suspended in 1 mL of 10% trichloroacetic acid, followed by 1 mL of distilled water and 40 µL of 0.1% ferric chloride ($FeCl_3$). All samples were analyzed using $OD_{700\,nm}$. An increase in absorbance is indicated by an increase in the reductive power of the tested extracts. The results were expressed as Means±SD of the measured OD_{700}. IC_{50} values were determined for each sample corresponding to the concentration required to reach 0.5 absorbance.

Evaluation of the antimicrobial properties

Biological material

Tubes containing 3 mL of BHI broth were prepared for the reactivation of *Staphylococcus aureus* B1 and *Listeria monocytogenes* ATCC19144. The tubes were incubated at 37°C for 24 hours with 100 µL of inoculum for each bacterial strain. Both bacterial samples were then seeded onto BHI agar plates and incubated at 37°C for 24 hours. A bacterial suspension was prepared from the incubated cultures using distilled water (10^8 CFU/mL). This is equivalent to the M^{c}Farland 0.5 standard.

Antibiofilm formation assay

This assay aims to study the ability of the test samples to disrupt the initial attachment of bacterial biofilms based on the protocol of **Bakrim *et al.* (2021)**. 100µL of *L. monocytogenes* suspensions (10^6 CFU/mL) were added to a sterile microplate. This was followed by the addition of 100 µL of each tested sample prepared in sterile BHI broth (50-12.5 mg/mL for *A. spinosa* oils and 5-1.25 mg/mL for Smen samples), sterile BHI broth was utilized for control. The plates were carefully sealed with parafilm to avoid contamination and incubated at 37°C for 24 hours while avoiding any sudden stirring that might potentially disrupt the adhesion of the cells to the surface of the plate. After incubation, the microplate

contents were carefully disposed of, and the microplate was rinsed with distilled water to remove non-adhered cells. The plates were dried for 1 hour at 50°C before introducing 200 µL of crystal violet (0.4%). After incubation for 15 minutes at ambient temperature, the wells were rinsed with more distilled water to remove any remaining stains. Subsequently, 150 µL of ethanol was added to each well, and 100 µL of this solution is transferred to a new microplate for absorbance measurements ($OD_{590\ nm}$). The following formula is used to determine the susceptibility of the cells to the antibiofilm action of the tested samples:

$$\mathbf{I}\ (\%) = \left(\frac{\mathbf{Abs_{Control}} - \mathbf{Abs_{Sample}}}{\mathbf{Abs_{Control}}}\right) \times \mathbf{100}$$

Analysis of bacterial kinetic

The bacterial growth kinetics of *S. aureus* and *L. monocytogenes* were analyzed to determine the influence of the test samples on bacterial growth parameters. The Biosan RTS-1 bioreactor was used to conduct the analysis. This system was particularly chosen since it allows for real-time growth analysis through a non-invasive strategy. This bioreactor is designed to provide a controlled environment for optimal cell growth. The RTS-1C's Reverse Spin technology involves the inverted rotation of the culture medium, which promotes the delivery of nutrients and oxygen to the cells while eliminating unwanted metabolites. An advanced control system that accurately monitors and regulates culture conditions is included with the RTS-1C. It is also designed to control contamination by incorporating filters into the tube caps. The testing procedure involved the addition of 2.5mL of the bacterial culture (10^8 CFU/mL) to TPP tubes containing 20 mL of MH broth inoculated with the active samples. The resulting suspension was placed in the bioreactor, and the latter was programmed at 37°C and 2000 rpm/min. The reverse tube spin period was set at 1s, and measurements were taken at 1h intervals for 24h **(Bakrim *et al.*, 2024)**. The bacterial growth rate and the generation time were calculated based on the generated data:

Bacterial growth rate: $\mathbf{(\mu) : Y_f = A_i e^{k\Delta t}\ (avec\ k = \mu)}$

Generation time (G)**:** $\mathbf{G} = \frac{\ln(2)}{\mu}$

Evaluation of antifungal activity

The antifungal effect of test samples was evaluated against *Fusarium oxysporum* by the plate diffusion method **(Wianowska *et al.*, 2016)**. On PDA plates, 1 mL of each sample was diluted in acetone (100 and 50 mg/mL). The acetone was then left to dry at room temperature. Mycelium discs (5 mm) were then removed from a week-long fungal culture and placed in the center of the agar. Plates containing pure acetone without test samples served as controls. The plates were incubated at 25°C for 4 days, and fungal growth was recorded daily. The percent inhibition of fungal growth (I%) was calculated using the following formula:

$$(\mathbf{I}\%) = \frac{\mathbf{Dc} - \mathbf{Dt}}{\mathbf{Dc}} \times \mathbf{100}$$

With:
Dc: Growth diameter of the control group
Dt: Growth diameter of the test group

Statistical analysis

All results were presented as Means ± Standard Deviation (SD). Statistical analysis on the obtained data was performed with IBM SPSS statistics V26 (IBM, Somers, NY, USA) using One-way ANOVA analysis along with post-hoc testing (Tukey's test). Significant differences between the concentration treatments and test samples are indicated by different letters ($P < 0.05$).

RESULTS

Fatty acids composition

The free fatty acid composition of both Argan Oil (AO) and Smen Oil (SO) is detailed in Table 1 below, which provides a detailed outline of the diverse and widely distinct FFA profiles obtained.
Both samples revealed distinctive profiles in terms of FFA percentage, AO mainly comprises unsaturated fatty acids, which represent around 80% of the total content, while saturated fatty acids represent only about 20%. Conversely, SO consisted of approximately 60% unsaturated fatty acids, while saturated fatty acids were nearly double AO, representing 40% of the overall profile.

Table 1 Fatty acids composition of the tested samples

	Oil samples				
Fatty acids (%)	**AA**	**AC**	**TS**	**HS**	**CS**
C14:0	0.137	0.141	13.632	8.527	11.114
C16:0	13.968	12.541	34.04	29.655	32.837
C16:1	0.117	0.069	0.884	1.896	1.724
C17:0	0.075	0.074	0.869	0.793	0.684
C17:1	0.022	0.22	0.194	0.404	0.326
C18:0	6.425	5.196	16.302	10.139	9.865
C18:1	48.478	47.927	13.386	31.045	23.640
C18:2	29.937	33.239	1.332	2.443	2.139
C18:3	0.076	0.087	0.485	0.568	0.406
C20:0	0.372	0.319	0.518	1.115	1.08
C20:1	0.393	0.386	-	-	-
ΣSFAs	20.977	18.271	65.361	50.229	55.58
ΣUFAs	79.023	81.928	16.281	36.356	28.235

SFA: Saturated Fatty Acids; **UFA:** Unsaturated Fatty Acids

Physico-chemical properties

The total phenolic and flavonoid contents in both *A. spinosa* oil samples and the examined Smen varieties are presented in Figures 1 and 2. According to the obtained data, TPC and TFC contents were detected in different amounts depending on the samples tested. Both tests appeared to present a similar variation trend between tested samples ($p<0.05$). TFC was detected in amounts ranging from 0.44 mg RE/g sample to 0.04 mg RE/g sample. Phenolics were detected in much higher amounts at an interval of 2.07 mg GAE/ g sample to 0.63 mg GAE/ g sample. HS revealed the highest TPC, yet it presented the lowest content of flavonoids on the other hand, its commercial counterpart (CS) proved to be the richest in TFC. As for argan oil, both samples demonstrated the lowest TPC content; however, the obtained values did not vary significantly from the Smen samples (CS and TS), and they equally presented moderate TFC values.

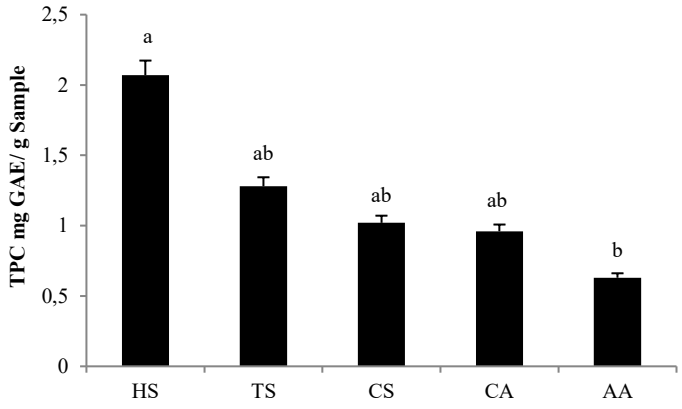

Figure 1 Total polyphenols content (mg GAE/g Sample)
*Means in the same column with distinct lettering (a–b) differ significantly at $p<0.05$. ** **AA**: Artisanal Argan; **CA**: Commercial Argan; **TS**: Tazrout Smen; **HS**: Had lgharbia Smen; **CS**: Commercial Smen.

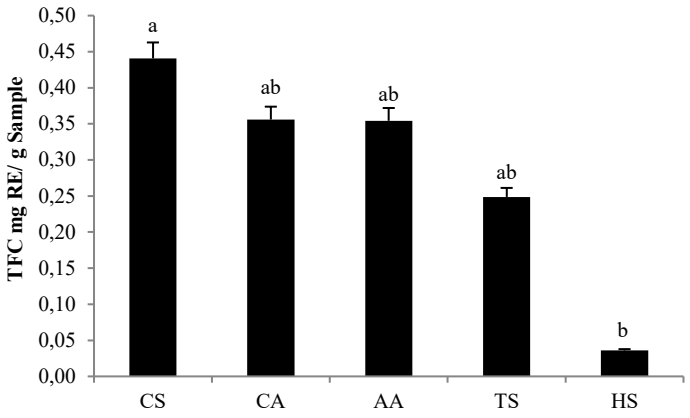

Figure 2 Total flavonoids content (mg RE/ g Sample)
*Means in the same column with distinct lettering (a–b) differ significantly at p<0.05. ** **AA**: Artisanal Argan; **CA**: Commercial Argan; **TS**: Tazrout Smen; **HS**: Had lgharbia Smen; **CS**: Commercial Smen.

The tested samples were studied to determine their antioxidant potential using a free radical scavenging assay and the ferric reducing power (DPPH and FRAP). Overall, the obtained data from both screenings were detailed in Tables 2 and 3, and Figures 3 and 4. Results reported in Table 2 indicate that argan samples displayed significant antioxidant power, with registered values of 92.2 to 33.4% and 82.89 to 8.68% for AA and CA, respectively. Both samples recorded a dose-dependent trend between the tested concentrations and antioxidant capacity ($p < 0.05$). A similar trend has been obtained while testing the Tazrout Smen (TS) sample (94.26 to 10.97%). As for the two remaining Smen samples (HS and CS), no antioxidant activity has been recorded. The FRAP assay registered higher antioxidant profiles, and it was revealed that both CS and HS presented a significant antioxidant potential in this system, with OD_{700} values of (0.682-0.237) and (0.768-0.229) for HS and CS, respectively, at concentrations ranging between 10 and 0.312 mg/mL, therefore, demonstrating an effect higher than that obtained with TS (0.561-0.199). As for the argan oil samples, CA was more active than AA, particularly at higher concentrations (10 mg/mL, 50 mg/mL, and 25 mg/mL). A significant difference was observed across all tested samples, indicating that the observed variations in antioxidant activity within each sample were mainly attributed to concentration ($p < 0.05$).

IC_{50} values have been calculated to standardize the results obtained for both test systems (DPPH and FRAP), which would allow for a better comparison. Table 4 summarizes the recorded values for each sample. It was found that the FRAP assay generated lower IC_{50} values, which indicates that the samples presented a higher level of activity/compatibility in this system than in the DPPH test. AA was the most efficient compound in the FRAP assay, with an IC_{50} value of 1.5 mg/mL followed by CS (4.25 mg/mL), HS (4.88 mg/mL), TS (7.76 mg/mL), and finally CA (15.6 mg/mL). Those values were lower than the ones recorded for the free radical scavenging assay except for TS, which reported a higher efficiency in DPPH than FRAP (5.48 mg/mL and 7.76 mg/mL for DPPH and FRAP respectively).

Table 2 Free radical scavenging ability of *A. spinosa* oil samples

A. spinosa	**Concentration**	**100**	**50**	**25**	**12.5**	**6.25**	**3.125**
	Artisanale	92.2±0.12^{a}	84.98±0.38^{b}	69.38±0.5^{c}	51.36±0.77^{d}	40.69±2.65^{e}	33.40±0.45^{f}
	Commercial	82.89±2.08^{a}	62.71±4.59^{b}	35.08±4.95^{c}	25±2.33^{c}	11.31±1.14^{d}	8.68±3.38^{d}

* Means in the same column with distinct lettering (a–f) differ significantly at p<0.05.

Table 3 Free radical scavenging ability of Smen samples

Smen samples	**Concentration**	**10**	**5**	**2.5**	**1.25**	**0.625**	**0.3125**
	Tazrout	94.26±1.54^{a}	32.96±0.96^{b}	20.8±0.38^{c}	16.84±0.19^{cd}	14.66±0.57^{de}	10.97±1.54^{e}
	Had lgharbia	NA*	NA	NA	NA	NA	NA
	Commercial	NA	NA	NA	NA	NA	NA

*: Non Active; ** Means in the same column with distinct lettering (a–e) differ significantly at p<0.05.

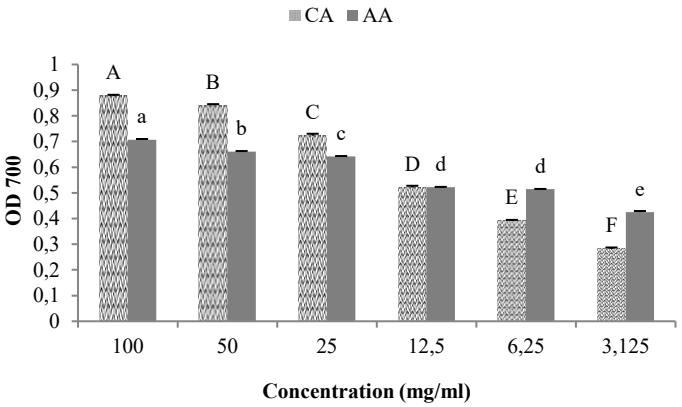

Figure 3 Ferric reducing capacity of *A. spinosa* oil samples
*Means in the same column with distinct lettering (a–f) differ significantly at p<0.05. ** **AA**: Artisanal Argan; **CA**: Commercial Argan.

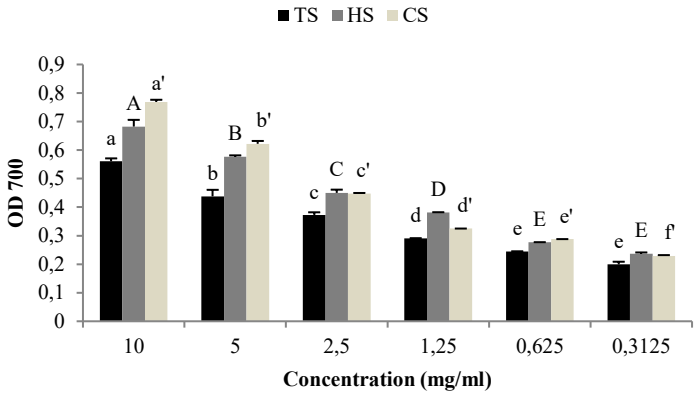

Figure 4 Ferric reducing capacity of Smen samples
*Means in the same column with distinct lettering (a–f) differ significantly at p<0.05. ** **TS**: Tazrout Smen; **HS**: Had lgharbia Smen; **CS**: Commercial Smen.

Table 4 Antioxidant efficacy of Argan and Smen samples (IC_{50})

Sample	DPPH	FRAP
AA	12.2	1.5
CA	48.88	15.6
TS	5.48	7.76
HS	-	4.88
CS	-	4.25

*__AA__: Artisanal Argan; **CA**: Commercial Argan; **TS**: Tazrout Smen; **HS**: Had lgharbia Smen; **CS**: Commercial Smen.

Evaluation of the antimicrobial properties

The effect of the five tested samples on the initial attachment and formation of *L. monocytogenes* biofilm is presented in Figures 5 (Artisanal and Commercial Argan) and 6 (Tazrout, Had lgharbia, and Commercial Smen). Overall, all tested samples presented high efficacy against *L. monocytogenes* biofilms, with the highest inhibition percentages belonging to HS, with an inhibitory activity estimated at 96.21 %, 95.86%, and 96.44% across the tested concentration range. At 5 mg/mL, the commercially available sample of Smen managed to successfully suppress *L. monocytogenes*' initial attachment by 91.84%, this activity persisted even at lower concentrations, recording inhibitory values of 86.3% and 86.1 %, slightly higher values were observed for the Tazrout sample (94.41%, 93.27%, and 89.95 %). Alternatively, Artisanal and Commercial argan oils recorded lower activity, however, both samples managed to exert a substantial restrictive effect reaching values above 70% (83.72%, 74.83%, 70.81%, and 91.95%, 85.74%, 82.55% respectively for AA and CA at 50 mg/mL, 25 mg/mL, and 12.5 mg/mL). Even though all samples revealed varying effects across the tested concentration range, it is noteworthy that these differences did not reveal any statistical significance ($p>0.05$).

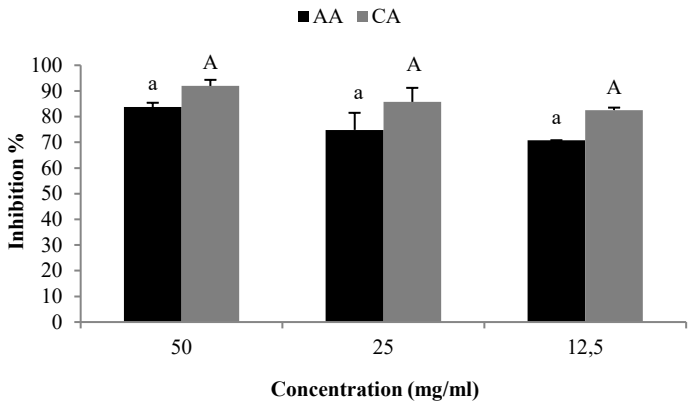

Figure 5 Effect of different concentrations (50-12.5 mg/mL) of *A. spinosa* artisanal oil (AA) and commercial oil (CA) on 24 h old *L. monocytogenes* biofilms.
*Means in the same column with distinct lettering differ significantly at $p<0.05$.
** **AA**: Artisanal Argan; **CA**: Commercial Argan.

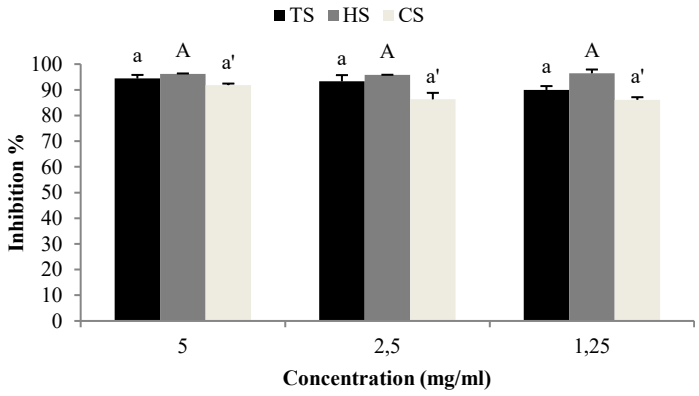

Figure 6 Effect of different concentrations (5-1.25 mg/mL) of Tazrout Smen (TS) Had lgharbia Smen (HS) and Commercial Smen (CS) on 24 h old *L. monocytogenes* biofilms
*Means in the same column with distinct lettering differ significantly at $p<0.05$.
** **TS**: Tazrout Smen; **HS**: Had lgharbia Smen; **CS**: Commercial Smen.

In regards to the influence of the tested Smen and *A. spinosa* oils on the bacterial kinetics (*S. aureus* and *L. monocytogenes*), a screening of the five samples was carried out using incubation experiments, and the bacterial growth parameters (growth rate and generation time) were calculated from the obtained growth curves. The results obtained are provided in Table 5. Notably, a considerable effect was obtained in response to the samples.
In the case of *S. aureus*, AA, CA, and CS demonstrated an inhibitory pattern of extended generation times compared to the control group, extending the doubling time nearly 6.4-folds for AA, 4.12-fold for CA, and 20.5-fold for CS (4.41 h, 2.84 h, 14.14 h, in comparison with the control: 0.69 h).
Conversely, the Smen samples obtained from both Had lgharbia and Tazrout regions remain the most active against the strain, these variations suggest a potential bacteriostatic effect, since upon incubation with *S. aureus*, a notable absence of bacterial development was observed across the incubation period. An absence of the typical bacterial growth phases —the exponential growth phase in particular — was equally noticed which prevented us from calculating the growth parameters.
Regarding growth rates, the data reveals a consistent trend across the tested groups. Incubation of *S. aureus* with each sample consistently resulted in lower growth rates compared to the control (0.163 h^{-1}, 0.287 h^{-1}, and 0.05 h^{-1}), indicating a potential suppressive effect on bacterial replication.
Meanwhile, *L. monocytogenes* presented a minor deviation in its generation time when incubated with AA, CA, and CS. With an extension rate reaching 3.3-fold, 8.6-fold, and 5.7-fold, TS managed to reveal the most significant effect, extending the generation time 26 times slower than the control, which is the highest rate observed across the screening, consequently reducing the growth rate to 0.04 h^{-1}, the lowest recorded growth rate for *L. monocytogenes*. In the meantime, HS continues to exert its bacteriostatic effect against *L. monocytogenes* as well.
For *L. monocytogenes* growth rates, a noted statistical significance was observed between AA and all samples, CA and HS, TS and CS, and HS and CS. While the generation time recorded a significant variance between TS and all tested samples. As for *S. aureus*, the obtained generation time for CS varied significantly from all observed samples, whilst the growth rates revealed no statistically significant variations ($p>0.05$).

Table 5 Bacterial growth parameters in the presence of Argan and Smen samples

Sample	*Staphylococcus aureus*		*Listeria monocytogenes*	
	μexp (h^{-1})	**G (h)**	**μexp (h^{-1})**	**G (h)**
Control	0.994	0.69	0.971	0.71
AA	0.163±0.04	4.41±1.1	0.299±0.05	2.35±0.42
CA	0.287±0.15	2.84±1.5	0.118±0.02	6.11±1.2
TS	NG*	NG	0.04±0.01	18.48±6.5
HS	NG	NG	NG	NG
CS	0.05±0.009	14.14±2.7	0.169±0.01	4.1±0.17

*: No Growth; ** **AA**: Artisanal Argan; **CA**: Commercial Argan; **TS**: Tazrout Smen; **HS**: Had lgharbia Smen; **CS**: Commercial Smen.

Figure 7, presented below, shows the percentage of mycelial growth inhibition of AA, CA, TS, HS, and CS at two different concentrations (100 mg/mL and 50 mg/mL) obtained by the agar diffusion method against *F. oxysporum*, a plant pathogenic fungus responsible for the development of wilt diseases in many crops. The data were obtained during a screening period of four days for each concentration. Overall, all screened samples showed some level of antifungal activity against *F. oxysporum*, as the percentage of inhibition surpassed 50% during the first 48 hours of incubation for all samples. CA exerted the highest antifungal potential at both concentrations, as it had the highest inhibitory rate across the entire incubation period. A similar effect was observed with CS, which closely followed CA in terms of fungal growth inhibition. As for artisanal samples, AA revealed a good antifungal potential, with values surpassing 50% during day 2, however, this effect continued to decline as the incubation period extended to day 4. Both TS and HS demonstrated similar effects at 100 mg/mL during day 2 with a percentage value of 62.86% and 61.43%. This trend in similarity persisted till day 4, where a clear difference was observed between the two samples (44.44% and 26.39%) which clearly indicated that the TS sample was the most active against *F. oxysporum*. This consistency in activity is maintained by TS even at a lower dose (36.11% of inhibition by day 4). The antifungal potential of the samples varied as a function of concentration and the compounds tested; however, statistical analysis revealed that neither the compound nor its concentration had a significant effect on the observed inhibition rates.

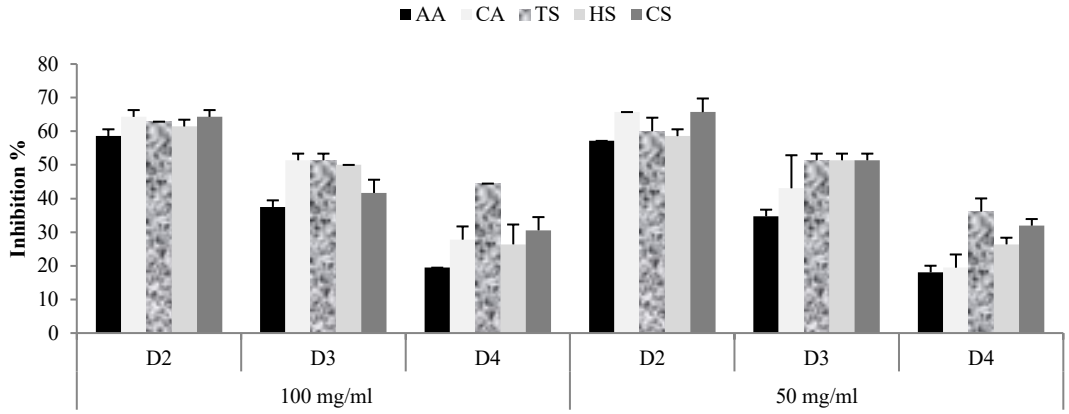

Figure 7 Antifungal potential of *A. spinosa* oil and Smen samples (100-50 mg/mL)
* **AA**: Artisanal Argan; **CA**: Commercial Argan; **TS**: Tazrout Smen; **HS**: Had lgharbia Smen; **CS**: Commercial Smen.

DISCUSSION

Fatty acids (FAs) stand as the fundamental constituents within vegetable oils, underscoring their pivotal role. It's worth highlighting that the characteristics, endurance, and nutritional significance of a specific vegetable oil are greatly contingent on the composition of its FAs (Gharby ***et al.,*** **2021)**. Table 1 provides an overview of the alterations in the fatty acid composition of both AO and SO, originating from various sources. AO primarily consists of approximately 80% unsaturated fatty acids, with saturated fatty acids accounting for only about 20% of its composition. On the other hand, SO contains roughly 60% unsaturated fatty acids and approximately 40% saturated fatty acids. The nutritional advantages of unsaturated fatty acids are extensively supported by documented evidence. AO is primarily characterized by its high content of oleic acid, comprising between 47.927 and 48.478%, along with notable levels of linoleic acid, which range from 29.937 to 33.239%. The profile obtained for AO is in accordance with previous studies profiling the FA content of Argan oil obtained from areas across the Souss-Massa region in Morocco (Essaouira-Taroudant-Agadir Idaw Tanane-Tiznite Chtiuka Ait Baha and Sidi Ifni), in this study the major component was found to be oleic acid (39-50%), followed by linoleic acid (27-39%), and palmitic acid (11-15%) **(Miklavčič *et al.,* 2020)**. Conversely, SO is distinguished by its significant quantities of palmitic acid, ranging from 29.655 to 34.04%, and oleic acid, which varies from 13.386 to 31.045%. The current findings align with prior studies that have assessed how the fatty acid composition changes in relation to the region of harvest: argan oil from Essaouira and Agadir **(Idrissi *et al.,* 2023)**, olive oil **(El Moudden *et al.,* 2020)**, and cactus oil **(Taoufik *et al.,* 2015)**.

Both the polyphenolic and total flavonoid contents ranged between 2.07 mg GAE - 0.63 mg GAE and 0.44 mg RE - 0.04 mg RE respectively (Figures 1 and 2). HS proved to be the richest in polyphenols, with the highest value obtained in the screening (2.07 mg GAE/g sample), followed by TS, Smen also proved to be the richest in flavonoids, with the commercially available sample (CS) having the highest content estimated at 0.44 mg RE/g sample. Therefore, it proves that the Smen samples are the most valuable source of secondary metabolites in comparison with argan oil. The importance of such findings relies on the fact that polyphenols in general are widely known for their biological activities, with reported benefits to the consumer's health, mainly being implicated in the reduction of inflammatory reaction, cardio-protective effects, as well as reducing the risks of cancer and UV radiation **(Tungmunnithum *et al.,* 2018)**. Therefore, following a diet containing Moroccan argan oils and Smen used either as cooking oils or for direct consumption could effectively contribute to the overall improvement of the consumers' health.

Both AA and CA demonstrated considerable activity in both DPPH and FRAP systems, the highest efficiency in the FRAP assay belonged to AA, this effect is mainly attributed to the chemical composition of argan oil which is mainly known to be rich in tocopherols, largely implicated in the antioxidant activity, as opposed to Smen, a fermented butter characterized with high content of free fatty acids mostly responsible and are reported to have an antibacterial effect **(Badreddine *et al.,* 2020; Desbois and Smith, 2010; Jing *et al.,* 2019)**. AA showed higher overall antioxidant potential in both systems compared to CA (IC_{50}=12.2 mg for DPPH and 1.5 mg for FRAP), this variation in potential could be explained due to the difference in the regions both samples were collected from. Plants tend to produce higher levels of polyphenols, flavonoids in particular, when exposed to various environmental stresses **(Afi *et al.,* 2024)**. Indeed, **Idrissi *et al.* (2023)**, demonstrated that oil samples from the Agadir region (more arid) have a higher antioxidant efficiency than Essaouira samples (higher humidity). However, for argan tree, other factors can intervene and stimulate the increase in polyphenol levels such as herbivore stress, radiation and temperature levels, and nutritional stress **(Afi *et al.,* 2024)**. Since the antioxidant effect did not correlate to the TPC content of the tested samples, it is most likely that polyphenols were not the only compounds responsible for the observed inhibitions, but rather due to the implication of other compounds also known for their antioxidant potentials, such as tocopherols, carotenoids, and sterols, most particularly in argan oils **(Seiquer *et al.,* 2015)**.

On the other hand, Smen (Ghee) was reported to be highly rich in free fatty acids and mainly known to have antibacterial **properties (Desbois and Smith, 2010)**. This observation is confirmed by the results obtained for the analysis of the initial bacterial cell attachment (Figures 5 and 6). Even at lower concentrations, Smen samples were capable of inducing a higher potential to prevent the attachment of *L. monocytogenes* cells and biofilm formation. HS represented the highest anti-biofilm potential (96.21% for 5 mg/mL), this effect remained relatively stable with no statistically significant discrepancies across the tested concentration range, closely followed by TS (94.41% at 5 mg/mL), AA was the least active sample, however, it still managed to inhibit 83.72% of total cell attachment at 50 mg/mL. It is important to note that this effect was obtained at a dose 10 times higher than that of Smen samples. According to our knowledge, our study is the first to report on the effect of Moroccan artisanal products (Argan oil and Smen) on *L. monocytogenes* biofilms, which could have a positive impact as a setting stone for a new research path regarding the control of *L. monocytogenes* associated infections and biofilm formation in food processing plants. Other studies, however, focused on the analysis of the inhibition of bacterial development through the broth microdilution assay, for the determination of the minimum inhibitory concentrations. For instance, **Lall *et al.* (2019)**, demonstrated that oil and saponin extracts stemming from *A. spinosa* leaves were capable of curbing the growth of *Cutibacterium acne*s with a MIC of 500 µg/mL, therefore supporting the application of argan for acne treatment. Similarly, **Bonvicini *et al.* (2017)** reported the efficacy of *A. spinosa* organic extracts in controlling *S. aureus, S. epidermidis*, *E. faecalis*, *E. coli*, *K. pneumoniae*, and *P. aeruginosa*'s growth. Other studies exhibited that Smen (ghee) can potentially curb bacterial survival **(Adamu and Sajo, 2021)**.

Similarly to the antibiofilm assay, the influence of Moroccan argan oil and Smen obtained from different regions of Morocco on bacterial kinetics is reported for the first time in this study. In a similar trend, Smen samples continued to exert a significant effect on the growth kinetics of both *S. aureus* and *L. monocytogenes*. According to Table 5, TS exerted total growth inhibition on *S. aureus*, while HS prevented the growth of both tested strains. As for CS, it demonstrated the lowest growth rate upon incubation with *S. aureus*. Analysis of bacterial kinetics in the presence of potential antibacterial elements is of particular importance in several fields since it allows for predicting bacterial behavior and how it may react in a given system and therefore allows for better control and prevention of contamination. Consequently, this study provides insight into the search for natural antibacterials of major interest, particularly in the food industry.

Figure 7 summarizes the antifungal potential of the five tested samples against *F. oxysporum*, a soil-borne pathogen largely responsible for vascular wilt disease in many crops (particularly date palm trees), which leads to significant yield losses **(Rahmouni *et al.,* 2019)**. *A. spinosa* oil and its leaves, as well as Smen (ghee), are widely used in Morocco for medicinal purposes and have been shown to have antimicrobial properties **(Adamu and Sajo, 2021; Bonvicini *et al.,* 2017; Lall *et al.,* 2019)**. Subsequently, this study can contribute to the advancements currently taking place for the development of natural and sustainable strategies for the control of *F. oxysporum*-related infections in crops, particularly since commercially available synthetic fungicides have been associated with several environmental and health hazards **(Komárek *et al.,* 2010)**. Each compound was tested at two concentrations (100 mg/mL and 50 mg/mL) and the percentage of mycelial inhibition was determined for day 2, 3, and 4 of incubation. The obtained data revealed that the inhibitory rate varies depending on the tested sample, its concentration, and the days of incubation. For instance, CA at 100 mg/mL had a higher inhibition on day 3 (51.39%) compared to day 4 (27.78%). Similarly, TS revealed a percentage of inhibition of 36.11% on day 4 at 50 mg/mL, an activity that is significantly lower than the one observed on day 2 for the same

concentration (60%). However, no significance in inhibition between the two concentrations or between the different compounds was detected, which means that although there are some variations in the data, neither concentration nor compound had a significant effect on the observed results. Overall, this investigation represents a novel inquiry regarding the biological properties of Smen and Argan oil, an aspect that remained lightly researched despite the high consumption rate by the Moroccan population. Therefore, could potentially contribute to the validation and valorization of these two products as well as stimulate further research for possible applications across various sectors.

CONCLUSION

Argan oil samples revealed a higher potency as antioxidants with their evident activity in both DPPH and FRAP, while Smen samples, on the other hand, were the most effective in creating unfavorable environments for *L. monocytogenes* biofilm formation and were able to induce bacterial growth inhibitions (HS) against *L. monocytogenes* and *S. aureus*. This antimicrobial potential was further evidenced by the samples' capacity to disrupt *F. oxysporum* development. In summary, artisanal products traditionally produced and used in Morocco presented significant biological activities. The data generated during the different test modules reflects the diversity in chemical components of the samples, which requires further investigation.

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