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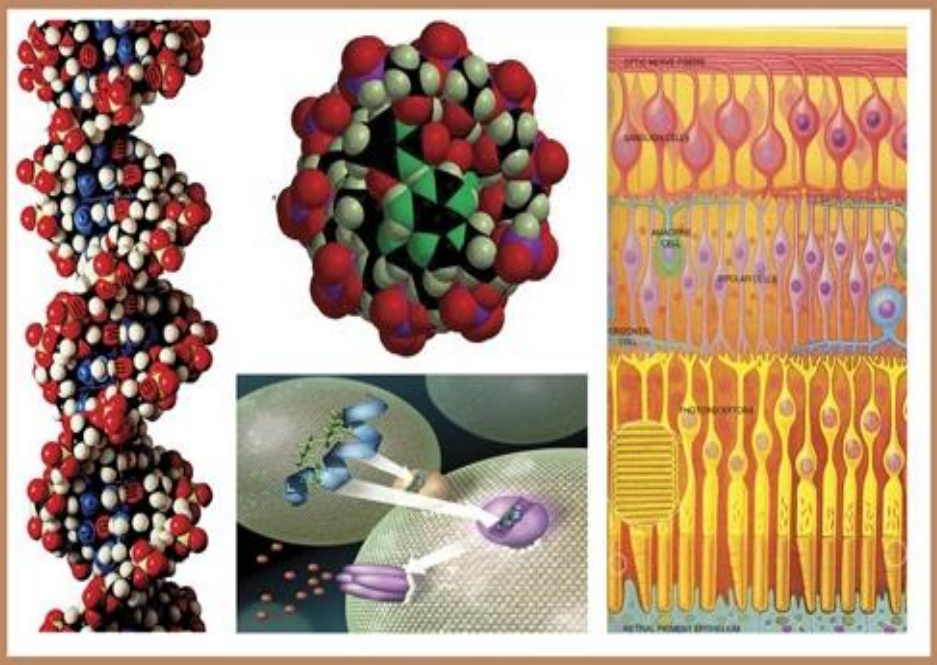

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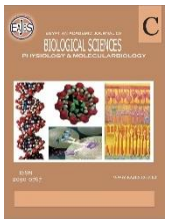

Estimation of the Stability and Preservation of Fresh Minced Beef Meat Enriched with *Linum Usitatissimun* Oil During Refrigerated Storage

Labbaci Fatima Zohra[1], El kadi Fatima Zohra[2], Ouramdane Refka[2], Hadjari Mohammed[3], Bentekhici Malika[2] and Sennous Kouider[4]

1-Department of Biology, Faculty of Nature and Life Sciences, University of Mustapha Stambouli, Mascara, 29000, Algeria.

2-Laboratory of Molecular Microbiology Health and Proteomics, Biology Department, Faculty of Natural Sciences and Life, Djillali Liabés University of Sidi-Bel-Abbés,22000, Algeria.

3-Geo-Environment and Space Development Laboratory, Research Unit on Microbial Biotechnology and Environment, Department of Biology, Faculty of Natural Sciences and Life, Mustapha Stambouli University, Mascara 29000, Algeria.

4-Spatial Ecodevelopment Laboratory, Department of Environmental Sciences, Faculty of Natural Sciences and Life, Djillali Liabés University of Sidi-Bel-Abbés,22000, Algeria.

*E. Mail: elkadifatimazohra@yahoo.fr

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ABSTRACT

The current study's main goal is to assess the antioxidant activity, secondary metabolites, and physicochemical quality indices of linseed (*Linum usitatissimum L.*) oily extract (*Luo*), along with the effect of linseed oil addition on the preservation of beef mince during refrigerated storage at 6°C. The oil was extracted by the Soxhlet method from *Linum usitatissimum* seeds with a yield of 53.96%, and its physicochemical and biological quality were assessed. The results showed that the oil met international standards, with values of refractive index (1.4764), acid index (0.40± 0.06 mg KOH/g), saponification index (177± 0.66 mg KOH/g), ester index (176.6 ± 0.66 mg KOH/g), and peroxide index (1.5 ± 0.66 meq d'O2/kg). Secondary metabolite analysis revealed that the concentrations of phenolic compounds, flavonoids, flavonols, and condensed tannins were (0.50±0.04mg GAE/ml oil), (0.39±0.02mg QE/ml oil), (0.038±0.003mg QE/ml oil), and (0.036±0.002 mg CE/ml oil), respectively. However, IC50 value was 0.54±0.06 mg/ml, demonstrated a remarkable ability to scavenge the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH-). Likewise, pH of the ground meat enriched with flaxseed oil was extremely high compared to the control meat. Ultimately, *Linum usitatissimum* oil was abundant in bioactive and antioxidant molecules. This suggests that flaxseed oil could be used to promote the food industry as a preservative.

INTRODUCTION

The food industry relies on the requirement to deliver two essential necessities: the preservation of aliments and the conception of products that are economical, constantly available, simple to prepare and providing all the guarantees of hygiene and harmlessness for the consumer (Hébel, 2010). Meat is a perishable foodstuff, and its quality alteration affect the consumer's health at risk, due to the development of pathogenic microorganisms responsible for food poisoning of various severities. In addition, lipoperoxidation is one of the main causes of deterioration in the quality of meat and products and generates compounds that can be detrimental to health such as malondialdehyde (MDA) (Michel *et al*., 2008).

Therefore, lipids deterioration provokes micronutrients deficit, nutritional value decrease, organoleptic qualities degradation, and even generates toxic substances (Nessrien & Mohamed., 2007). Flaxseed is an oleaginous seed produced by the cultivated flax (Linum usitatissimum L.) (Linaceae). The whole seeds can be employed to make flaxseed oil and animal meal, or they might be consumed for their health benefits including constipation relief and improved cardiovascular markers. They are currently the plant raw material with the highest concentration of Phyto-estrogenic lignans1 and omega-3 fatty acids. They are also renowned for their mucilage content (Lamblin *et al.,* 2008).

Meat preservation is a public health issue attributable to the widespread use of synthetic antioxidants such as nitrates, nitrites, and sulfites, which are toxic to consumers (El kadi *et al.,* 2014; El kadi *et al*., 2017; El kadi *et al.,* 2021), hence the need to substitute them with bio-conservatives.

The goal of the current work is to investigate the physical, chemical, and biological properties of *Linum Usitatissimun* and its oil extract, as well as the effect of its addition to fresh bovine meat (FBM) stability.

MATERIALS AND METHODS

Biological Components:

Flax seeds, *Linum usitatissimun* procured from the local store in Sidi Bel Abbes, *Linum usitatissimun* were washed and dried outdoors and then broyed, placed in an opaque container and kept at room temperature in the dark. Likewise, FBM was purchased from a local market in Sidi Bel Abbes, FBM was preserved at 4°C until use. The rationale for choosing this product was the meat's susceptibility to microbiological changes and its widespread consumption by the Algerian population.

Physicochemical Analysis of *Linum usitatissimum* Powder:

Flaxseeds were tested for moisture, fat, ash, titratable acidity, pH, Na+ and K+, and crude fiber (AOAC 2010).

Furthermore, macronutrient estimation was accomplished by determining total proteins using the Bradford (1976) method, fat content (NF ISO 8262-3, 2006) and total carbohydrates were estimated using the following formula:
Total carbohydrates (g/100 g) =100− (m fat+ m ash+ m proteins) (Bazile *et al.,* 2016)

Oil Extraction (Soxhlet method):

Linum usitatissimum oil **(***Luo*) recovery was based on the extraction of lipids from flaxseeds by the addition of an organic solvent (hexane) using the Soxhlet apparatus.

The Macroscopical Observation of *Linum usitatissimum*:

Macroscopically oil analysis was conducted in three steps: visual (aspect and color), olfactory-gustatory (composition, quality, and intensity of flavors), and tactile (onctuosity).

Physicochemical Characteristics of *Linum usitatissimum:*

In order to assess the purity and commercial quality: physicochemical characteristics were established, namely: Refractive index (RI); Acidity index (AI); Saponification index (SI); Ester index (EI); Peroxide index (PI) (AOAC, 2010).

Secondary Metabolites Quantification:

Phenolic Extract Process:

1ml of n-hexane and 2ml of 60% methanol were added to 2gr of oil. The mixture was agitated before being centrifuged (3000 rpm /5 min). The supernatant has been retrieved. The procedure was repeated three times to extract the oil, which was then combined and concentrated under vacuum at 40°C from becoming reconstituted in 1ml of 50% methanol. (Pirisi *et al.,* 2000).

Phenolic Compounds Content (Folin-Ciocalteu):

The spectrophotometric Folin-Ciocalteu reagent method was used to estimate the total phenolic contents according to (Vasquez Roncero *et al.,* 1973) technique, briefly,

A mixture of 500µl of Folin-Ciocalteu reagent and 450µl of distilled water were added to 50µl of phenolic extract. The mixture was shaken vigorously, thereafter 400µl of sodium carbonate Na2CO3 (7.5%) was incorporated. At 725 nm, the optical density was measured. The values were reported as mg gallic acid equivalent per kg of oil (mg GAE/ml oil)

Flavonoid Content:

Flavonoid content estimation was performed following the (Kim *et al.*, 2003) method, a mixture of 0.4 ml of distilled water, 0.03 ml of sodium nitrite (5%) and 0.02 ml of aluminum chloride (10%) was added to 0.1ml of extract. After incubation for 5 min at room temperature, 0.2ml sodium hydroxide (1M), and 0.25ml distilled water were added. Absorbance was measured at 510 nm; flavonoid content was expressed as mg catechin equivalent per kg oil (mg EC/ml oil)

Flavonol Content:

A total of 0.5 ml of aluminum chloride (2%) and 0.75 ml of sodium acetate (5%) were mixed with 0.5 ml of the phenolic extract. After 30 min incubation at room temperature, absorbency was read at 440 nm, the results were given as mg quercitrin equivalent per kg oil (mg EQ/ml oil) (kumaran *et al.*, 2007).

Condensed Tannin Content:

To 0.25 ml of extract was added 2.5 ml of iron sulfate solution diluted in HCl and butanol [FeSO4 + HCl-butanol (2:3)]. The mixture was incubated at 95°C for 50 min. The absorbance was measured at 530 nm (Skerget *et al.*, 2005). The results are evaluated in accordance as follows: C= (A530×mm×df)/εL. Whose, A530= the absorbance of the extract at 530 nm; MM=287.24g/mol (molar mass of cyanidin-3-glucoside); ε =34700 l.mol-l.cm-1 (the molar extinction coefficient of cyanidin-3-glucoside); Fd= dilution factor; L=optical path; C: concentrations were represented in mg equivalent of cyanidin-3-glucoside per ml of oil.

Anti-Free Radical (DPPH) Assay:

The DPPH assay was performed following the method described by Tepe *et al.* (2005), a concentration series of extract was prepared in methanol. 10 µl of each was added to 1ml of a methanolic solution of DPPH (0.004%). After 30 min of incubation at 25 °C, the absorbance was read at 517nm. The inhibition of DPPH free radical by the percent decolorization (I%) of DPPH is evaluated by the formula:

I % = [(Abs blanc – Abs échantillon) / Abs blanc] x 100.

IC_{50} was obtained from the curve of extract concentrations in relation to I %. This parameter was defined as the concentration of phenolic compounds required to decrease the initial DPPH concentration by 50%.

Influence of *Linum usitatissimum* Oil (*Luo*) Addition on Meat pH:

Samples (10gr) of fresh beef hash (FBH) were processed as follows:

1. Control group (GC): FBH (without Luo enrichment)
2. Group 1 (gr 1): FBH + 20 µl of *Luo*
3. Group 2 (gr 2): FBH + 40 µl of *Luo*
4. Group 3 (gr 3): FBH + 60 µl of *Luo*

The four samples were kept in the refrigerator at 4°C for 5 days. Furthermore, 3 grammes of FBM from each group was mixed with 27 ml of distilled water, shaken for 30 min with a magnetic stirrer, then filtered, the filtrate obtained was the subject of the pH determination using a pH meter, this operation was repeated 3 times for 5 days.

Statistical Analysis:

The results were expressed as mean standard errors (X±SE). Statistical analysis of the data was conducted using Microsoft Excel version 7.0 software. In all cases, a value of $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

Physicochemical Evaluation Powder:

The outcomes of the physicochemical analysis of the flax grain powder are illustrated in (Table 1).

Table 1: *Linum usitatissimun* physicochemistry evaluation

Parameters	Values
Water (%)	6.5 ±0.08
Titratable acidity	50 ±0.66
pH	6.74±0.01
Ash (%)	2.7 ± 0,22
Fat (%)	41.78 ±1.10
Total protein (%)	20.06 ±0.90
carbohydrate (%)	29.02 ± 0.06
Crude fiber (%)	33.60 ±0.02
Na^+ (mg/l)	7.80 ±0.002
K^+ (mg/l)	200 ±0.004

Flaxseeds should be stabilized once at humidity of 7 to 8% to avoid acidification caused by insect and moisture evolution (Cetion, 2008). However, the humidity levels of our samples remain decreased than those found by (Berglund et Zollinger, 2007) which were 8%, and are closer to those found by Coskuner et Karababa, (2007) with a humidity level of 6%. The low water amount is detrimental to insects, particularly acariens, and to ensuring good conservation over a long period.

The pH of food determines its ability to be preserved. It constitutes one of the most complex obstacles that the microbial flora must overcome in order to thrive (Brissonet *et al.*, 1994). The pH value in our case was slightly higher than that found by Mihoubi (2019) (6.13). This suggests that the flaxseed had a slightly acidic pH.

Likewise, the cendre rate found in our study (2.7 0.22%) is too close to the values found by (Coskuner & Karababa, 2007) (3%–4%).

For macromolecules, our *Linum usitatissimum* samples contained levels of (20.06 ±0.90%; 29.02±0.006%; 41.78 ±1.10%) protein, carbohydrates and lipids respectively. These results are in agreement with the scientific literature, namely the work of Tatiana *et al.* (2017) (20.86% protein), and Coskuner and Karababa, (2007) with values of (20-25%) protein and (30-40%) lipid.

Crude fiber belongs to the insoluble fibers existing in the edible part of the cell wall of plants. They are essentially cellulosic compounds obtained as a residue from the chemical analysis of plant substances. The *Linum usitatissimum* powder studied showed a high crude fiber content (33.6%) that was higher compared to (Coskuner and Karababa) values (20-25%) (Coskuner and Karababa, 2007).

In addition, the mineral salts Na+ and K+ were lower than those found by the Ciqual database of Anses with (20.5 m/l; 641 mg/l) of Na+ and K+ respectively.

Macroscopic Aspect and Quality Index of The Oily Extract:

Based on the analysis of our test, *Luo* had an oily appearance, homogeneous, yellow color and faint odor characteristic of flaxseed (Table 2).

Table 2: Macroscopic characteristics of *Linum usitatissimun*

Properties	Aspect
Color	Golden yellow
Aroma	Assez prononcée
Appearance	Relativement fluide et homogène
Taste	Fairly pronounced
Yield	53.96 %

Following Soxlet extraction, flaxseeds presented a yield of 53.96% was relatively higher than that achieved by Amrouche (2013) 39.96%. In addition, the oil corresponded to the international standards international standards (codex alimentarius) (Table 3).

Table 3: Chemical characteristics of *Linum usitatissimun*

Index	Values
Refractive Index at 20 C °	1.4764
Peroxide value (meq d'O2/kg)	1.5 ± 0.66
Indice d'acide (mg KOH/g)	0.40 ± 0.06
Saponification value (mg KOH/g)	177 ± 0.66
Ester Index (mg KOH/g)	176.6 ± 0.66

The refractive index (IR) is another criterion of oil purity. The index depends on the chemical composition of the oil. Generally, IR increases according to the introduction or existence of secondary products (Karleskind, 1992). *Luo* IR value was in agreement with that of Mirela Popa *et al.* (2012) and Amrouche (2013) and *codex alimentarius* at values of 1.469, 1.4594, and (1.4720-1.4750) respectively. Fat index acid (IA) is a good indicator to determine its alteration by hydrolysis, *Luo* of the current study presented an IA of 0.40±0.06 mg KOH/g that remained decreased in comparison to that obtained by (Amrouche,2013) (3.08). Moreover, the saponification index (SI) was related to the chain length of the fatty acids constituting the oil. SI was 177±0.66 mg KOH/g oil, was lowered in relation to (Amrouche, 2013) and (Mirela Popa *et al.*, 2012) 191.1 and 190 respectively, furthermore it was in the range of the *codex alimentarius* standard (187 and 197).

In addition, the ester index (EI) was 176.6±0.66 (mg KOH/g), and peroxide index (PI) permits to identify oils state oxidation and control the initial stages of oxidative alteration. IP is related to storage conditions and extraction methods. The value of IP in this study was about 1.5±0.66 meq O2/Kg of oil, this value was lower than that found by Amrouche, (2013) 4.74 meq O2/Kg of oil.

Secondary Metabolites and Antioxidant Activity:

The biological activity of HGL was assessed by estimating secondary metabolites and antioxidant activity.

Table 4: Secondary metabolites and antioxidant activity of *Linum usitatissimum*

Secondary metabolites	Values
TPC (mg GAE/ml oil)	0.50±0.04
TFC (mg QE/ml oil)	0.39±0.02
Flavonols (mg QE/ml oil)	0.038±0.003
CTC (mg CE/ml oil)	0.036±0.002
IC50 mg/ml	0.54±0.06

Each value was expressed as means ± Standard deviations for triplicate experiments. CE: Catechin equivalent; GAE: Gallic acid equivalent; QE: Quercetin equivalent; TFC: Total flavonoid content; TPC: Total phenolic content; CTC: Condensed tannins content

The results of the current research confirm the richness of our oil extract in secondary metabolites having significant antioxidant activity. Moreover, our values remained lower in comparison to Alachaher, (2018) and Acket, (2015) results.

In fact, the phenolic composition varies between plants in the same species, this may be related to climatic factors, genetic heritage, the stage of development of the plant and its degree of maturation, the period of its harvest, the duration of storage, the method of extraction and the method of quantification of compounds of biological interest (Bouzid, 2009).

Supplementation of Fresh Beef Hash with *Linum usitatissimun:*

According to our analysis, the FBH (not enriched with luo) was acidified during the 5 days of storage, whereas the FHBV enriched with an increasing concentration of *Luo* maintained a basic pH during the 5 days of storage, with a proportional relationship being established between the pH and the concentration of *Luo* (dose/effect).

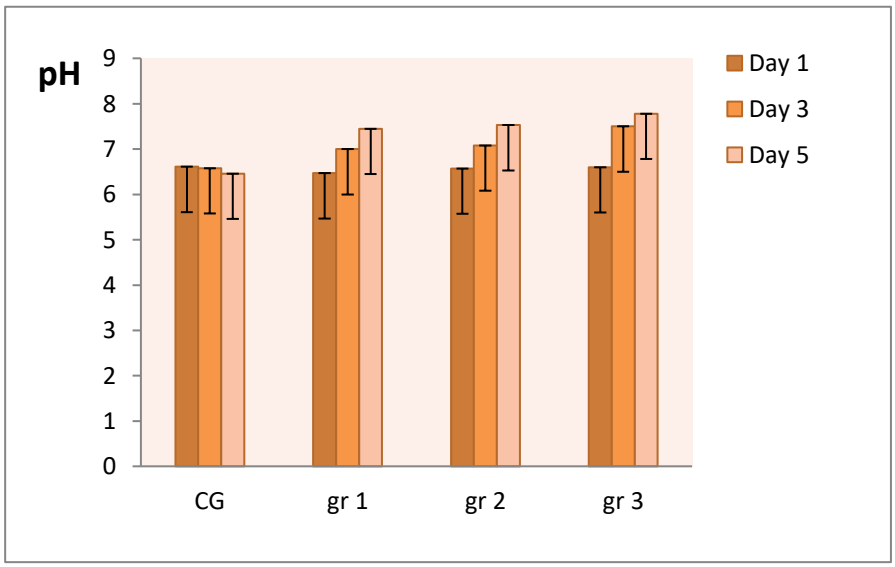

Fig. 1: *Linum usitatissimum* oil effect on the pH variation of ground beef stored at 6°C for 5 days.

The pH value is extremely important for the meat, as it has a decisive influence on the quality criteria. From the pH value, it is possible to judge the color, tenderness, taste, water retention and conservation time. The biochemical decomposition processes in the meat start after slaughter that have an influence on the pH value. PH-value measurement provides information about the speed processes and meat quality. Our outcomes confirmed a stable pH value of *Luo*-enriched fresh beef hash during storage. The elevation in pH could be related to the decarboxylation of amino acids by the microorganisms (Yasin and Abou-Taleb, 2007). Therefore, current work indicates that *Luo* has a strong preservative power, given its richness in antioxidant molecules.

According to the literature, there are no studies conducted on the preservation of meat by *Luo*. Nevertheless, several works confirm the preservative power of some plants and vegetables, namely: Amany *et al.*, (2010), Ayari *et al.*, (2016), which confirmed the significant elevation in average pH values of all meat samples treated with essential oils of garlic, thyme and lemon at all concentrations during 6 days of conservation. In addition, Skandamis and Nychas, (2000) confirmed that the addition of oregano essential oil to ground meat increases the pH values during storage.

CONCLUSION

The results of the present work show that the extract of vegetable oil of Lu obtained by soxhlet presents a good quality and may be considered a promising antioxidant preservative agent for the food industry. This study reinforces the use of *Linum usitatissimun* oil as an antioxidant

conservator in the agricultural sector.

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