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THÈSE

Présentée par

ARAB-MOULOUD Radia

Pour l'obtention du grade de

DOCTEUR EN SCIENCES

Filière : Biologie

Option : Sciences Alimentaire

Thème

Incorporation d'une graine oléagineuse dans un produit de transformation alimentaire: Cas des graines de sésame dans le yaourt

Soutenue le : …….....

Devant le Jury composé de :

Nom et Prénom	Grade		
Mr MADANI Khodir	Professeur	Univ. de Bejaia	Président
Mme BOULEKBACHE Lila	Professeur	Univ.de Bejaia	Rapporteur
Mr FREIDJA M. Lamine	MCB	Univ. de M'sila	Co-rapporteur
Mme HAMRI Sabrina	MCA	Univ. de Bejaia	Examinateur
Mr KADRI Nabil	MCA	Univ. de Bouira	Examinateur
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Année Universitaire : 2019/2020

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Scientific Research
A. MIRA University, BEJAIA
Faculty of Natural and Life Sciences
Department of Food Sciences
Laboratory or research unit attached to it: Laboratory of
Biomathematics, Biophysics, Biochemistry and Scientometry (L3BS)

THESIS

IN ORDER TO OBTAIN THE DIPLOMA OF DOCTORATE

Sector: Biology
Specialty: Food Science

Presented by
ARAB-MOULOUD Radia

Theme

Incorporation of an oilseed in a food processing product: Case study sesame seeds in yogurt

Supported on: ……. **In front of the Jury composed by:**

Name and First Name	Grade		
Mr MADANI Khodir	Professor	Univ. of Bejaia	President
Mrs BOULEKBACHE Lila	Professor	Univ. of Bejaia	Reporter
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University year : 2019/2020

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*****

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Dedication

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I dedicate this modest work To my dear parents who have always supported me, especially my mom: you are and you will remain for me the symbol of kindness, the source of tenderness and my source of courage and strength For their love, patience and encouragement

*****

To my dear littles Aylane and Willas

*****

To my dear sisters and brother this work is also their work

*****

To my husband and his family

*****

RADIA

Scientific publications

1. **QUALITY PARAMETERS, PROBIOTIC VIABILITY AND SENSORY PROPERTIES OF PROBIOTIC STIRRED SESAME YOGURT.** Radia Arab, M. Lamine Freidja, B. Dave Oomah,Sonia Benali, Khodir Madani, Lila Boulekbache- Makhlouf. The Annals of the University Dunarea de Jos of Galati Fascicle VI – Food Technology (2020), 44(1): 9-26.

2**. Seed roasting affect fatty acid profile ,lignan, sterol and tocopherol content and oxidative stability of sesame oil**. Radia Arab[1*], Susana Casal[2],Teresa Pinho[2], Rebeca Cruz[2], Mohamed Lamine Freidja[1,3], Khodir Madani[1,4], Lila Boulekbache-Makhlouf[1] (Submitted and revised).

Participation in scientific events

1. ARAB Radia*, Effect of roasting on sesame lipids and quality. Lila BOULEKBACHE-MAKHLOUF, CASAL Susana and MADANI Khodir. 1st Doctoral Day of Natural and Life Sciences. December 16, 2019. University of Bejaia, Algeria.

2. ARAB Radia*, Lila BOULEKBACHE-MAKHLOUF, CASAL Susana and MADANI Khodir. Sesame oil: impact of roasting on lignans content, quality characteristics, composition and oxidative stability . International seminar on local products: a tool for the development of Mountain Agriculture, 15 and 16 December 2018. Chemini, Bejaia, Algeria.

3. ARAB Radia*, Lila BOULEKBACHE-MAKHLOUF, CASAL Susana and MADANI Khodir.. Sesame lignanes enhance auxidative stability of vegetable oils . 2nd National Exhibition of Research Products. Exhibition Centre, Pins Maritimes -SAFEX- Algiers from 02 to 04 July 2018, Algeria.

4. ARAB Radia* and BOULEKBACHE-MAKHLOUF Lila. Graine et tourteau de sésame (*sesamum indicum* L): composition chimique et apport en nutriments. 1st NATIONAL COLLOQUIUM "Society, Science, Sport, Health and Nutrition". 09 May 2018, Bejaïa, Algeria.

5. ARAB Radia* and BOULEKBACHE-MAKHLOUF Lila. Incorporation d'une graine oléagineuse dans un produit de transformation : étude de cas de graine de sésame dans un yaourt au probiotique. International symposium of microbial research. 02 and 03 May 2018, university of Khemiss Meliana Algeria.

6. ARAB Radia* and BOULEKBACHE-MAKHLOUF. Valeur nutritionnelle des graines de sésame (*sesamum indicum* L) et activité antioxydante de l'huile extraite. National seminar: sustainability at the service of our food, health and environment. 15 and 16 Janury 2018, Bejaïa, Algeria.

7. ARAB Radia*, BOULEKBACHE-MAKHLOUF, Nadjat BOUCHARA, Feriel Kermiche and MADANI khodir. Effet de la torréfaction et de la délipidation sur les composes bioactifs et l'activité antioxydante des graines de sésame (*sesamum indicum* L) . National Exchange Seminar: At the Interfaces of Sustainable Development. 20 and 21 December 2017, Bejaïa, Algeria.

Organizations

1. Participation in the organization of an international seminar "Local products: a tool for the development of Mountain Agriculture". 15 and 16 December 2018, Chemini, Bejaia, Algeria.

2. Participation in the organization of a national seminar "National Exchange: At the Interfaces of Sustainable Development 2017". 20 and 21 December 2017, Bejaia, Algeria.

List of abbreviations

***p*-AV :** Para anisidine value

AOAC : Association of Official Analysis Chemists

AV: Acid value

BB12: Bifidobacterium animalis

CFU: Colony forming unity

Ch-Cysteine: Cysteine cçhlorohydrate

D: Density

DG: Diglycerides

DPPH : 2, 2-Diphenyl-1-Picrylhydrazyl

EEC : European Economic Community

FA : Fatty acids

FAMEs : Fatty Acids Methyl Esters

FAO: Food and Agriculture Organisation

FFA : Free fatty acid

FID : Flame ionization detector

FOS: Fructo-oligosaccharides

Fru: Fructose

Gal: Galactose

GAE: Gallic acid equivalent

GC: Gas Chromatography

GC/MS : Gas Chromatography/Mass Spectroscopy

Glu: Glucose

GOS: Gluco-oligosaccharides

HDL : High-density lipoprotein

HPLC-RI : High Performance Liquid Chromatography-Refractive Index

HPSEC: High performance size exclusion chromatography

IP: Incubation period

ISO : International Organisation for Standardisation

IV: Iodine value

K232: Specific extinction or absorbance in the ultraviolet at 232 nm

K268: Specific extinction or absorbance in the ultraviolet at 268 nm

LB: Lactic bacteria

LDL *:* °Low-density lipoprotein

MRS: Man Rogosa Sharpe

MUFA: Monounsaturated fatty acids

OPA: o-phthaldialdehyde

Ox-TGM : Oxided triglyceride monomers

P: Probability

PUA: Polyunsaturated fatty acids

PY: Probiotic yogurt

RI: Refractive index

RSSO: Roasted sesame seed oil

SEC-HPLC : Size Exclusion-High Performance Liquid Chromatography

SFA: Saturated fatty acids

SPE : Solid phase extraction

SS: Sesame seeds

SSO: Sesame seed oil

ST : Solution test

SV: Saponification value

SY: Sesame yogurt

TC: Total carotenoids

TF: Total fat

TG: Triglycerides

TGD: Triglycerides dimmers

TGP : Triglycerides polymers

THF : Tétrahydrofurane

TPC: Total phenolic content

TTA: Total titratable acidity

UFA: Unsaturated fatty acids

USSO: Unroasted sesame seed oil

UV: Ultra violet

W/V: Weight/volume

List of tables

List of figures

Table of contents

Table of contents

III. Probiotics and prebiotics

Experimental part

Chapter I: Effect of roasting on biochemical composition, quality and oxidative stability of sesame seeds and oil

II. Impact of sesame addition on quality parameters, probiotic viability and sensory properties of stirred sesame yogurt

General introduction

General introduction

"Open sesame", the famous phrase from the Arabian Nights, reflects the distinguishing feature of sesame seed pod, which bursts open when it reaches maturity. Historically, an old text, the Thebes Medicinal Papyrus (1,552 B.C.), found in Egypt, described the medicinal effect of sesame seed as a source of energy and health. Hippocrates in Greece noted its high nutritive value. A Chinese book (300 B.C.), described sesame as a nutrient food having various physiological effects, a tranquil frame of mind, and preventing aging (Bi et al., 2005).

Sesame (*Sesamum indicum* L.) is the first oil seeds cultivated by humans and one of the world's most important crops (Uzun et al., 2007). Seeds are a potential source of nutrient, as they contain 35-57% oil, 20-25% protein, 20-25% carbohydrate and 5-06% ash. Their oil content is higher than most of the known oilseeds (Hwang, 2005).

Sesame oil is regarded as a high-priced and high quality product. It belongs to oleic-linoleic acid and contains approximately 14% saturated, 39% mono-unsaturated, and 46% poly-unsaturated fatty acids (Toma et al., 1979). Sesame oil have long shelf life compared to other vegetable oils, even when stored at ordinary ambient air temperatures (Bedigian & Harlan, 1986). It resists to oxidative rancidity despite its high degree of unsaturation (Crews et al., 2006). This is attributed to endogenous antioxidants which are mainly γ-tocopherol and phenolic derivatives (lignans): sesamol, sesamolin, and sesamin (Arendt *et al.*, 1986).

Sesame is generally consumed after roasting even for the production of oil. The compounds contained in oil have multiple physiological effects, such as estrogenic activity, providing anti-inflammatory functions, decreasing blood lipids levels, and increasing anti-oxidative ability and γ –tocopherol bioavailability (Hirata *et al.*, 1996; Kita *et al.*, 1998).

Roasting is a key step in sesame industry, that causes chemical changes and influences several characteristics and properties of produced oil. The impact of seed roasting conditions on the seed and oil quality and composition has been already studied. Biochemical composition, oxidative stability and the antioxidant factors responsible for the stability of roasted sesame seeds is highly affected by the conditions of the roasting process. Therefore, in order to make good products, it is important to establish the effect of roasting on quality of sesame seeds and oil (Yen *et al.*, 1986; Yen, 1990; Yoshida, 1994).

In recent years, there has been interest in the consumption of plant-based foods still growing among consumers. This social phenomenon is certainly linked to the price of

awareness of the cause and effect relationship between food quality and health (Allane and Benamara., 2010). Yogurt is the most known and consumed fermented milk with high nutritional value and health promoting ingredients (Allgeyer *et al.*, 2010). The increase in per capita annual consumption of yogurt can be attributed to the diversification (probiotic or symbiotic yogurt, light or defatted yogurt, fruit yogurt and liquid yogurt drinks) and availability of the low priced product in the market. The perception of the prophylactic and therapeutic properties of probiotics is the origin of consumption accumulates for fermented milk products, in particular yogurt (Mercenier et al., 2002).

Prebiotics and probiotics have experienced a revival of interest over the past decade, materialized by an explosion of scientific publications and underpinned by the partnership with the food industry. Their use, based on empirical observations, is more rational today. Probiotics are often lactic acid bacteria (lactobacilli, bifidobacteria) which exercise a beneficial action on the health of the host which ingests them by improving the balance of its intestinal flora (Fuller, 1991).

Probiotics are non-digestible substances that induce a beneficial physiological effect on the host by specifically stimulating the growth and / or activity of a limited number of bacterial populations already established in the colon like bifidobacteria (Gibson and Roberfroid, 1995). However, the probiotic effect of bifidobacteria depends on their survival rate not only in food but also in the gastrointestinal tract (Shah, 2000). Among the probiotic strains, *B. animalis subsp. lactis* (BB12) is the most studied and documented in the literature with over 200 scientific publications (Garrigues *et al.*, 2010). It can withstand harsh conditions compared to other strains and hence its common use in probiotic foods.

The beneficial effects of sesame seeds are of great interest for the conception of healthy dairy product such as probiotic stirred yogurt. Sesame could therefore be a very good source of growth factors and prebiotic components for yogurt and other foods containing probiotics, offering the possibility of improving the formulation of fermented milks from a nutritional point of view.

The current study aimed to determine:

- The effect of seed roasting temperature on sesame seeds and lipids. For this purpose, quality, composition, oxidative stability and antioxidant capacity of the seeds and extracted oil were assessed at different temperatures (150,180, 210,250 and 300°C).
- The impact of ground raw and roasted sesame seeds on stirred yogurt quality parameters (pH, titratable acidity and syneresis) and probiotic growth, (ii) evaluate

the acceptability of sesame yogurt by consumers comparing roasted and unroasted sesame yogurts.

For this, the present work will be divided in two parts:

- The first includes the bibliographic study, which is subdivided into three sections:
  - General information on Sesame seeds (*Sesamum indicum* L),
  - Sesame seed oil: extraction, use and nutritional value,
  - Probiotics and prebiotics.
- A second experimental part which focused on two aspects:
  - Effect of roasting on biochemical composition, quality and oxidative stability of sesame seeds and oil,
  - Impact of sesame addition on quality parameters, probiotic viability (*Bifidobacterium animalis* ssp. *lactis*, BB-12) and sensory properties of stirred sesame yogurt.

Literature review

I. Sesame seeds (Sesamum indicum L)

I.1. Origin and botany

Archaeological records indicate that sesame has been known and used in India for more than 5000 years; it is recorded as a crop in Babylon and Assyria some 4000 years ago (Weiss 1971) .It has been described as the oldest oil crop in the world and considered the queen of oil seeds (Bedigian et al., 1986). Sesame is believed to have originated in Africa; the very ancient cultivation of sesame extends to the warm and moderately humid areas of the world. It is cultivated mainly in the tropical and subtropical regions of Asia, Africa and South America (Terrones, 1990). Proof of the interspecific species, hybridization and photochemical analysis indicate that the ancestor of sesame occurred in the Indian subcontinent (Bedigian et al., 1986).

Sesame (Sesamum indicum L), is an annual herbaceous plant of the Pédaliaceés family, presenting a wide diversity of genotypes (Weiss, 2000; Söğüt, 2008). This plant has a cycle of 75 to 135 days according to the varieties. Sesame is 0.5 to 2.5 meter tall under optimal growing conditions. The stem, of square section longitudinally grooved, is green, rarely purple. It can be hairless, velvety or hairy. This hairy feature of the stem and twigs allows grouping of varieties (Kafiriti and Deckers, 2001). The lower leaves are opposite, broad (12 x 8 cm), roughly lobed and long petiole (5 cm), on the other hand, the upper leaves are alternate, and narrow (9 x 2 cm) (Weiss, 2000; Kafiriti and Deckers , 2001).

Its flowers are yellow, but they are also found in shades of blue, white and purple in some cultivars. The seeds are enclosed in the fruit, which is a tiny, oval pod about 3 mm long (Anilakumar, 2010). A single capsule of the plant can contain up to 200 seeds. These, elongated and flattened, are very small, the weight of 1000 seeds varies between 2 and 3.5 g only, they can be black, brown, white or cream (figure 1), those of light color have higher oil contents, black seeds having thicker shells (Tashiro, 1990).

Figure 1: Plant, fruit and sesame seeds (Lim, 2012)

I.2. Nomenclature and classification

The species *S. indicum* is also known by the synonym *Sesamum orientale* L. Other lesser known synonyms such as *S. edule, S. luteum, S. oleiferum, S. africanum* and *S. foetidum* can also be cited (Demol et al., 2002).

The sesame also carries different nomenclature according to the regions: sim-sim (السمسم) or jenjlen in Arabic; sesame, bennissed, been (English); til (Hindu); gingelly (Iran); ajonjoli (Spain) and gergelim, sésamo (Portugal) (Hassan 2012). The botanical classification of sesame established by Guignard (2007) is given in table I:

Table I: Botanical classification of *Sesamum indicum*

Botanical classification of *Sesamum indicum*	
Reign	Vegetable
Division	Magnoliophyta
Class	Magnoliopsida
Order	Scrophulariales
Family	Pedaliaceae
Genus	Sesamum
Species	*Sesamum indicum*. L.

I.3. World production

Production of sesame seeds is increasing worldwide. Sesame ranks ninth among the top thirteen oilseed crops that make up 90% of the world's edible oil production (Saha *et al*., 2014). Around 3 million tones of sesame are produced each year worldwide, this quantity is cultivated in an area of more than 6.5 million hectares (Saydut et al., 2008; FAO, 2005; Rajeswari *et al.,* 2010). Most of it is harvested in Asia and Africa, i.e. 64 and 31% respectively of world production (Dada and Adeparusi, 2012). The main producers are India, Sudan, China, Burma, Nigeria, Ethiopia and Iran (Dawodu et al., 2014). Table II shows the major producers and the quantities produced in 2005.

Table II: Principal producers of sesame (FAO, 2005)

Country	Area harvested (h)	Production (tons)
China	660	800
India	1850	750
Myanmar	1370	606
Sudan	1700	331
Uganda	210.8	121
Nigeria	165.1	83
Pakistan	135.2	75
Ethiopia	93.1	72
Bangladesh	80.1	55
Central African Republic	42.1	47
Thailand	63.9	46
Tanzania	104.8	45
Egypt	29.9	41
Guatemala	55.8	39
Chad	95.1	39
Paraguay	67.9	37

I.4. Use of sesame seeds

Sesame is the oldest condiment used by humans for 1600 years BC (Anilakumar, 2010). There are many foods with sesame as an ingredient. The various uses of sesame have been enlisted in table III.

In human food, sesame flour is used in baking (preparation of bread and cakes), in Middle Eastern pastries, in the production of crisps and cookies, in exotic restaurants and vegetarian dishes. It is recommended in case of celiac disease because of its gluten deficiency (Moneret et al., 1999).

Sesame seeds add a nutty taste and a delicate, almost invisible crunch to many Asian dishes. Flour is the main ingredient in "*tahini*"(sesame seed paste) and the wonderful Middle Eastern sweet called "*halvah*" which is famous and well known. It is prepared by mixing sesame flour with glucose syrup, citric acid and other ingredients such as the aroma and fermented extract of *Saponaria officinalis* (Mohdaly et al., 2011).

Table III. Culinary uses of sesame seeds (Anilakumar, 2010)

Food	Country
Sesame cakes wine and brandy	Biblical Babylon
Bread stick, cracker, salad and cooking oil	Worldwide
Raw, powdered and roasted seed	India
Substitute for olive oil	Europe
Bread	Sicily
Cakes	Greece
Soup, spice and seed oil	Africa
Salad and fish oil	Japan
Confectionery	China
Sesame seed buns, chips	United States

Whole roasted seeds have a sweet nutty flavor, are often added to various preparations based on wheat flour such as bread, croissants and crackers. In most East Asian countries, roasted black sesame seeds are often used as an additive to dishes made with rice (Namiki 1995). In the United States, the famous Mc Donald brand imports it from Mexico to use it in burgers pine. In Togo and neighboring countries the seeds are used to prepare vegetable soup (Anilakumar 2010). After oil extraction, the oil cake obtained (contains between 35 and 50% protein, rich in methionine and tryptophan) as well as defatted or residual flour (56 to 60% protein) are used for poultry feeding and livestock (Choi et al., 2008).

I.5. Composition and nutritional value of seeds

The seed is renowned for its very high nutritional value. It contains between 35-57% of excellent quality oil, rich in essential fatty acids. Proteins represent 19 to 25% of the weight of the seed. Carbohydrates and ash represent 20 to 25% and 05 to 06% respectively (Nascimento et al., 2012). The overall composition of the seeds is shown in Table IV.

Sesame seeds are an excellent source of minerals such as calcium and copper. They are also rich in phosphorus, iron, magnesium, manganese, zinc and vitamin B1 (Hassan et al., 2013). 100 g of seeds contains 3 times more calcium than 100 ml of cow's milk. This amount provides 100% of the recommended daily allowance (RDA) for manganese and potassium,

between 57 and 65% of the RDA for phosphorus and iron, and between 13 and 35% for zinc, calcium and copper (Anon, 2006).

Table IV: Average nutrient composition of sesame seeds per 100 g (Namiki, 1995).

Composé	**Quantité**
Energy (calories)	578
Moisture (%)	4 ,7
Protein (g)	19,8
Fat (g)	51,9
Carbohydrate (g)	15,3
Fiber (g)	3,1
Ashes (g)	5,2
Ca (mg)	1200
P (mg)	540
Fe (mg)	9,6
Na (mg)	2
K (mg)	400
Vit A (UI)	0
Carotenoïds (µg)	17
Vit B1 (mg)	0,95
Vit B2 (mg)	0,25
Niacine (mg)	5,1
Vit C	0

As shown in table IV, sesame seeds contain a significant amount of vitamin B. Since the vitamin B is contained in the coat or hull of the seed, hulled sesame seed contains no vitamin B (Brito and Nunez, 1982). In order to utilize vitamin B, it is necessary to use sesame seed flour or mashed whole sesame seed. Among the vitamins in seed, the presence of vitamin E is very interesting in relation to the effectiveness of sesame seed as a healthy food.

A recent research has been carried out in Morocco and has highlighted other mineral elements on sesame genotypes such as selenium (Se) and potassium (K) (Rizki et al., 2017). According to these authors, selenium, considered to be very beneficial for consumers, is very

present in the mineral composition of sesame seeds, like calcium and phosphorus; this reinforces the pharmaceutical and nutritional importance of sesame seeds.

The flour obtained from sesame seeds after oil extraction, contains proteins in high quantities, rich in sulfur amino acids (methionine and cystine) while many vegetable proteins have low sulfur amino acid contents like the protein of corn flour (Brito and Nuñez, 1982), soy (Iwe et al., 2001), milled rice and wheat flour (Peter, 2007). The average amino acid composition is summarized in table V.

Table V: Amino acid composition of sesame seeds (Anilakumar, 2010).

Compound	Quantity
Amino acid (g/ 16 g N)	
Thréonine	3,1-3,7
Valine	3,9-4,6
Cysteine + methionine	2.8-4.8
Isoleucine	4.0-4.2
Phenylalanine + tyrosine	6.4-9.6
Histidine	2.7
Tryptophan	1,3-1,5
Lysine	2.6-2.7
Argenine	12.0
Leucine	6,5-7

I.6. Effect of roasting treatment on sesame seeds

Roasting is a key step in the preparation of sesame oil, which leads to major changes in its biochemical composition, organoleptic properties and quality (Yoshida 1995). Roasting is the action of heating, grilling so as to transform the raw material into a product transformed under the effect of the couple associating a temperature gradient and the roasting time. The temperature / time combination leads to varying degrees of roasting (Yen, 1990).

Roasting process catalyzes many chemical reactions in nuts and seeds that significantly modify their chemical composition, and consequently the composition of oil extracted from them (Durmaz and Gokmen,2010). Figure 2 summarizes the most important chemical changes or reactions that occur in seeds during roasting.

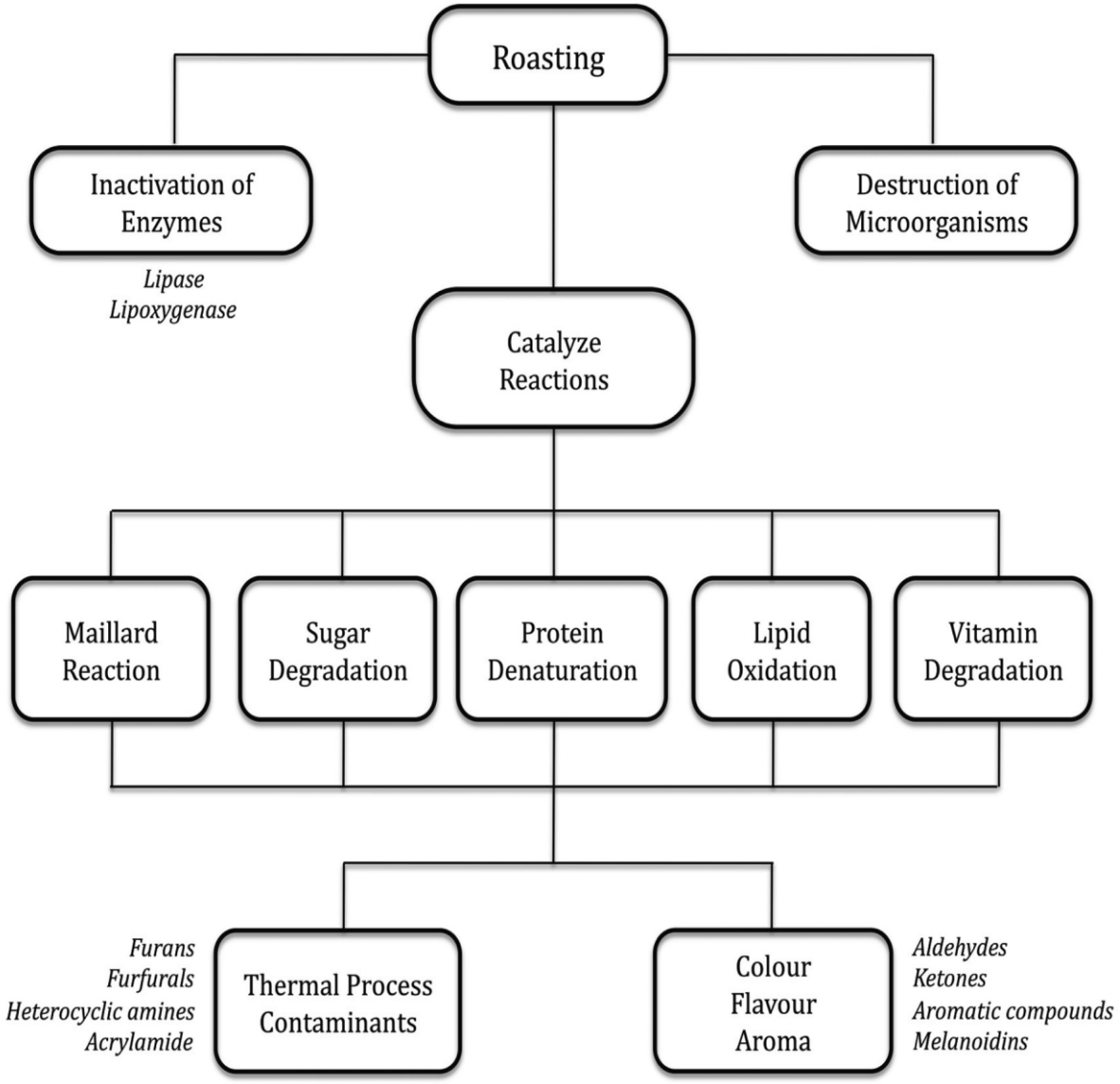


Figure 2: Summary of chemical reactions occurring generally in seeds induced by roasting (Durmaz and Gokmen,2010).

I.6.1. Effect of roasting on physical properties

The seeds lose up to 20% of their weight and their density can decrease up to 300 kg / m^3 (Dutra et al., 2001).

Color is an important quality criterion for roasted products. Roasting gives a sweet nutty flavor and a pleasant taste; it leads to a brown color which is a result of the products of the Maillard reaction (Kim et al., 2000).

Roasting can involve controlling seed moisture and heating conditions. The moisture content of the seeds after washing conditions can be controlled to create a better seed and oil quality for later storing (Mohammed and Hazma, 2008).

I.6.2. Effect of roasting on chemical changes

Sesame roasting process can be carried out with the conventional method, in muffle ovens or in electric ovens. The temperature and roasting time vary depending on the type of seeds (white, brown or black) and the type of oil that is sought. This process causes both positive and negative impacts on the quality (Kumar et al., 2009).

Roasting promotes the formation of potentially beneficial compounds, sesamolin is degraded into sesamol (figure 3), another phenolic compound which has been tested *in vitro* and *in vivo* and which has shown a very important antioxidant power and beneficial properties on humans, especially after the work of Hirata et al. in 1996 (Kumar et al., 2009, Anilakumar et al 2010). During the hydrogenation and refining of sesame oil, sesamolin is also transformed into sesaminol by a rearrangement reaction (figure 3).

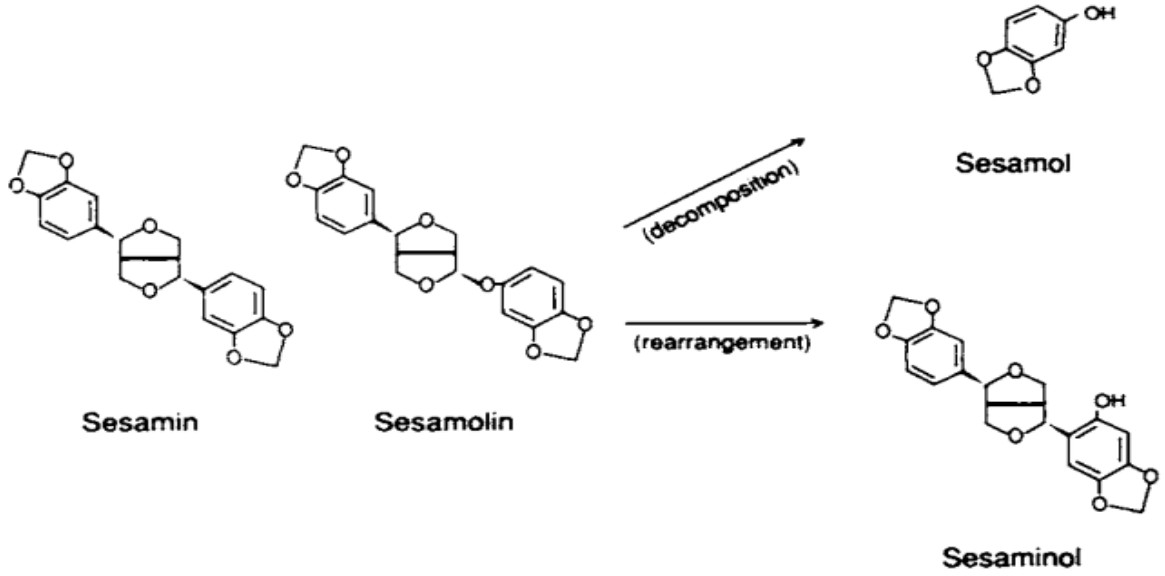


Figure 3: Decomposition and rearrangement reactions of sesamolin (Anilakumar et al., 2010)

Many studies were carried out on the roasted sesame flavor and more than 400 components have been isolated and identified. They have been classified as pyrazines, pyridines, pyrroles, furans, thiophens, thiazoles, carbonyl compounds, and others. Among them, alkyl pyrazines are the main components and provide the representative deep-roasted flavor, whereas

thiazoles and thiophenes are assumed to contribute to the characteristic roasted sesame flavor. In addition to the pleasant aroma of the roasted sesame flavor, the antithrombosis effect of these flavor components has been investigated and discussed further on (Shimoda et al., 1997; Shahidi et al., 1997; Namiki, 1998, Namiki, 2007).

Meanwhile, roasting can also induce the loss of nutritionally important macromolecules including proteins, vitamins, phenolic compounds, sterols and carotenoides.

- **Maillard reaction**

The Maillard reaction is a complex reaction having as reagents the reducing sugars and the amino acids or amides (Machiels and Istasse, 2002). The concentrations of reducing sugars and amino acids, moisture content, temperature and time are effective parameters affecting the kinetics of browning of nuts and seeds. Usually, there is linear correlation between color and roasting degree although all Maillard reaction products do not having dark color. The loss of lysine, which is an essential amino acid, is used as a marker for the progress of the Maillard reaction in thermally processed foods (Durmaz, G. et al., 2010).

- **Sugar degradation**

Caramelisation is another reaction in which sugars are involved during roasting. Sugar pyrolysis is the main driving factor for caramelisation in which amino acids are not involved. Compared to the Maillard reaction, caramelisation requires higher temperatures during roasting. The amount of simple sugars usually decrease and polysaccharides such as starch may be partly hydrolyzed releasing reactive sugars available for further reactions during roasting (Durmaz, G. et al., 2010).

- **Protein denaturation and enzyme inhibition**

Proteins are denaturated and this is important from the viewpoint of the activity of naturally occurring enzymes in sesame seeds. This treatment inactivates enzymes that are relatively less resistant to heat. Oil-degrading enzymes, lipase and lipoxygenase are partly or completely inactivated depending on the temperature and time of heating.

The acid value which provides information on the content of free fatty acids in the oil (due to the enzymatic activity) after roasting can be reduced due to the enzymes destruction with high temperatures which makes it possible to prolong the shelf life of this oil (Nzikou et al., 2009).

- **Newly formed compounds**

Recent studies had reported that potentially toxic compounds in foods during thermal processing are released. 'Acrylamide' is a possible human carcinogen formed in heated product as a result of the Maillard reaction between asparagine and reactive carbonyls at temperatures higher than 100°C. Sesame seed among other oily seeds and nuts contains certain levels of asparagines which make it susceptible for the formation of acrylamide during roasting. However, acrylamide does not pass to the oil phase during extraction due to its highly polar nature.

I.6.3. Effect of roasting on sesame oil

The oil obtained from roasted sesame has a pleasant color, odor and taste. Roasting provides a nutty aroma and roasted taste due to the several reasons. Maillard reaction products, lipid and protein oxidation and degradation products, caramelisation products are some of these factors. Pyrazines, a group of compounds formed at final stage of the Maillard reaction, are thought to be responsible for nutty aroma in extracted oil (Durmaz and Gokmen,2010).

The impact of seed roasting conditions on the quality of the oil has already been studied (Yen et al.,1986; Yen, 1990; Yoshida, 1994). Roasting increases the amount of oil extracted (Park & Kang, 2004); increases the concentration of lignans and antioxidant activity (Mohamed & Awatif, 1998; Yoshida *et al.*, 2000).

High roasting temperature can lead to the production polar compounds like oxidized polymers and trans fatty acids. It also causes the release of free fatty acids by hydrolysis of mono-, di- and triacylglycerols. The release of free fatty acids can accelerate the oil oxidation process, resulting in poor oil quality (Jeong et al., 2004).

Another significant consequence of the roasting process is thermoxidation of polyunsaturated fatty acids (PUFA) that leads to the formation of reactive carbonyls (RC) having varying volatility. These RC, are called as secondary lipid oxidation products, may also contribute to the progress of the Maillard reaction during the roasting process (Durmaz and Gokmen,2010). The sensitivity of unsaturated fatty acids is higher in phospholipids than that of the triglycerides when roasted was carried at higher temperatures (Yoshida and Takagi 1997).

Phytosterols such as ß-sitosterol are known to decrease slightly with roasting. Primary and secondary lipid oxidation products (hydroperoxydes and aldehydes/ketones, respectively) are enhanced during the heat treatment. The p-anisidine and thiobarbituric acid values are used to indicate the progress of lipid oxidation during roasting (Yaacoub, et al., 2008).

II. Sesame seed oil: extraction, use and nutritional value

II.1. Production and yield

The plant has a significant potential for the production of seeds rich in fat (42-56%), this yield is obviously a determining factor since it is higher than the range indicated for several oil seeds such as sunflower, canola, palm (30 to 50% crude oil) and soybeans (18 to 20%), considered a main source of edible oil for several years (O'Brien, 2009; Nadeem and Imran, 2016). This explains why it is one of the oldest seeds cultivated by humans (from 2500 years BC in Mesopotamia, Syria and Palestine). The total amount of oil produced in the world was about 1,900,000 tons in 2002. This amount is small compared to other oil seeds such as soybean, rapeseed, and oil palm, but the value of sesame seed and oil is considerably higher (Namiki, 2007).

II.2. Extraction

Several techniques exist for the extraction of oil which varies according to the regions and depending on the finished product. Sesame oil is one of the few vegetable oils that have been proven to be used or consumed directly without refining (Sudhir et al. 1996).

Further extraction with a solvent gives sesame flour of good nutritional value with low oil content. Shelling is common in India, where sesame flour is an important food and can be done manually at the village level or mechanically in conventional oil mills. In East Asia (China, Japan, Korea), where sesame oil is the main product, whole seeds are roasted, ground and cooked before extraction. In northern China, the seeds are soaked, roasted, shelled before being ground into sesame paste, and the oil (called small mill sesame oil) is separated by centrifugation or gravitation (Hwang, 2005).

In some African sesame-producing countries, extraction at the farm is done by grinding with a millstone and adding boiling water to remove the oil, resulting in poor quality crude oil. A more developed process uses manual presses and 3-stage extraction: the first extraction is carried out at room temperature and produces high quality oil; the cake obtained is heated and pressed to give a colored oil which is then refined; a third stage gives an inedible oil (Mkamilo *et al*., 2007). The sesame oil flour, generally obtained from unhulled seeds, is used to feed livestock and poultry (Hansen, 2011; Oplinger et al., 1990).

II.2.1. Factors that influences quantity and quality of extracted oil

The variety of plant, the climate, the type of soil and the stage of maturation of the seeds are the major factors which affect in faith the composition and the quantity of sesame seed oil (Akinoso et al., 2010). The applied pressure, the heating temperature, the heating time, the moisture content, seed size and the conditions of seed storing are the major parameters which influence the yield and the quality of vegetable oil (Weiss, 2000). The seed can undergo treatments before extraction such as shelling, cooking and roasting. If the seeds are not roasted, the oil is very light in color, odorless and if the seeds are roasted then the oil would be light or dark brown in color (depending on the intensity of the treatment applied) with a pronounced taste like hazelnuts (Weiss, 2000).

II.2.2. Extraction methods

Cold extraction and solvent extraction are the most frequently used methods for obtaining sesame seed oil. In addition, the researchers tested different extraction methods; traditional and innovative, chemical and conventional (green) to determine their efficiency and profitability in oil, while ensuring the use of this oil for human consumption. Other processes are also used: aqueous enzymatic extraction and supercritical carbon dioxide extraction.

II.3. Interest and use of sesame oil

The use of vegetable oils has seen a huge increase in the prevention or treatment of many diseases. *Sesamum indicum* oil has long been considered a natural source of linoleic acid, which reduces serum total cholesterol and low density lipoprotein (LDL) levels, and regulates the HDL / LDL ratio thus minimizing the risk of cardiovascular diseases (O'Brien, 2009).

Sesame oil is characterized by its high oxidation stability due to the presence of endogenous antioxidant substances such as sterols, tocopherols and lignans (sesamin, sesamolin and sesamol) (Were et al., 2006).

Sesame oil is a staple in the diet of many Asian countries. It is used in human food for seasoning and frying, in Japan it is used for cooking fish and making margarines and shortenings (Anilakumar *et al.*, 2010). In China, it is used in traditional medicine for preventing skin aging, and for the treatment of burns and wounds (Shenoy *et al.*, 2011).

Its price is higher than that of other vegetable oils (Sun, 2013), but many are its other outlets: traditional medicine and pharmaceutical industry (Terrones, 1990). In industries, it is

used in the manufacture of beauty products, lotions, soaps and oils for baths and massage (Moneret et al., 1999). Furthermore, it is used to make paints, insecticides and biodiesel (Asghar et al., 2014).

In traditional medicine, sesame oil is used in the prevention of cardiovascular diseases; it protects the liver, reduces cholesterol and has an antidepressant effect (Terrones, 1990). In some countries, it is used to treat hemorrhoids and stomach ulcers (Chopra et al., 1986). It is used for its laxative, emollient and softening character. It is also used to treat skin burns caused by the sun. It has also been shown to be effective in removing lice from children's tangles (Anilakumar 2010).

II.4. Chemical composition of sesame oil

II.4.1. Fatty acids

The oil content of sesame seeds is higher than most of the known oil seeds (Hwang, 2005). The oil contains a high amount of polyunsaturated fatty acids (PUFA),it is classified in 4th position in the PUFA content after the sunflower oil, soybean and corn (Oplinger et al 1990). Sesame oil is considered an expensive, high-quality product. It belongs to the “oleic-linoleic” category and contains approximately 14% saturated fatty acids, 39% unsaturated fatty acids and 46% polyunsaturated fatty acids (Toma and Williams, 2000).

The predominantly saturated fatty acids are palmitic and stearic acid with contents varying from 9.1 to 10.4 and 3.2 to 5.9% respectively (Wereet al., 2006). The fatty acid composition of sesame oil is shown in table VI.

Table VI : Fatty acid composition of sesame seed oil (Namiki, 1995)

Fatty acid	Content in %
12 :0	0.29
14 :0	0.14
16 :0	9.4
18 :0	4.76
20 :0	0.58
16 :1	0.30
18 :1	39.1
18 :2	40
18 :3	0.46
20 :1	0.21
22 :1	0.39
Saturated fatty acids	15.2
Monounsaturated fatty acids	39.99
Polyunsaturated fatty acids	40.46

II.4.2. Unsaponifiable matter

The unsaponifiable fraction represents 1% to 1.5% approximately and contains a varied and diversified class of compounds. Despite their small quantity, the minor compounds (unsaponifiable matter) of *Sesamum indicum* play an essential role in the fight against several pathologies. Sesame oil contains natural antioxidants which contribute as cardio and hepato-protective agents (Olatosin et al., 2014). There is a beneficial relationship between antioxidant activity and the hepato-protective effect of seed oil, which justifies its use in traditional medicine since ancient times (Anilakumar et al., 2010).

II.4.2.1. Tocopherols

Tocopherols are compounds that act as a non-specific antioxidant in the chain and prevent the spread of lipid oxidation (O'Brien, 2009) by stabilizing hydroperoxyl and other free radicals. The different isomers of tocopherols show different antioxidant activities in the in vivo and in vitro systems: the α- and γ-tocopherol isomers appear to be the most powerful antioxidants in food systems (O'Brien, 2009; Tsao and Li, 2008).

The tocopherol content of sesame is lower than that found in some vegetable oils such as soybeans and corn. The major tocopherol in sesame is γ-tocopherol (figure 4) which represents 97% of the total tocopherols, on the other hand the content of α-tocopherol is very low (Speek et al.,1985). The antioxidant activity of γ-tocopherol is higher than that of α-tocopherols in vitro, but the efficiency of γ-tocopherol in the physiological action of vitamin E is 10 times less than that of α-tocopherols (Speek et al. 1985). Effective synergy was observed between γ-tocopherol and sesame lignans, resulting in a very significant oxidative stability of sesame oil when stored under heat and light (Fukuda, 1981).

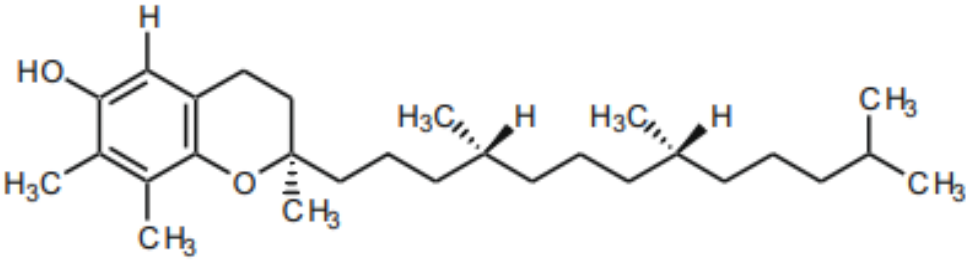


Figure 4: Structure of γ -tocophérol (Rangkadilok et al. 2010)

II.4.2.2. Sterols

Sterols are steroidal compounds that differ in their number of carbons and in the presence or absence of double bonds (Botelho et al., 2014). Phytosterols are a subgroup of the steroids, as an important class of bioorganic molecules, widespread in plants.The sterol content of sesame is relatively higher (> 8000 mg / kg) than that found in other edible vegetable oil such as olive oil, sunflower, soybeans and peanuts (Kamm et al., 2001). Sesame oil is rich in β-sitosterol and campesterol (figure 5), but this oil is particularly rich in Δ-5 -avenasterol, known for its anti-polymerizing properties when the oil is subjected to high temperature in frying and refining (Blekas et al., 2011).

The consumption of sesame sterols have been reported to reduce low density lipoprotein (LDL) cholesterol levels (Weststrate and Meijer, 1998). Among phytosterols, β-sitosterol wish is abundant in sesame seed oil, is used in the treatment of heart disease, hypercholesterolemia, modulating the immune system, prevention of rheumatoid arthritis and cancer, tuberculosis, hair loss and benign prostatic hyperplasia (Saeidnia et al., 2014).

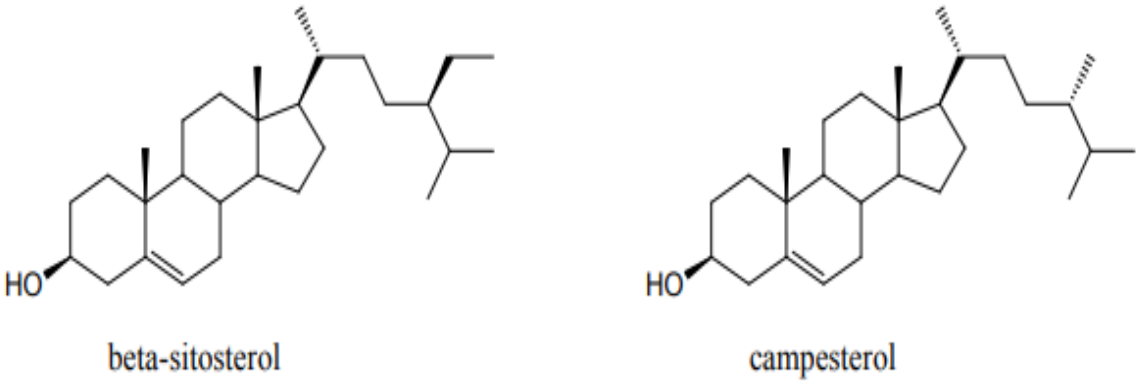


Figure 5: structure of the two major sesame sterols: β-sitosterol and campesterol (Saeidnia et al., 2014)

II.4.2.3. Lignans

Lignans are a large group of natural compounds which are defined as an oxidative coupling product of *β*-hydroxyphenylpropane. They are the major phenolic compounds squeezed into sesame oil with relatively high quantities (29.331 g / 100 g oil) (Saha et al., 2014). Some lignans are known to have antitumor, antimitotic, and antivirus activities. Interestingly, sesame seed contains significant amounts of characteristic lignans such as sesamin, sesamolin, sesaminol and others, as shown in figure 6.

Lignans are classified as phytoestrogens because they can mimic some effects of estrogen. These compounds can also have anti-estrogenic activities. Once administered, they are metabolized to enterodiol and enterolacton by the intestinal flora (Bi, et al., 2005). The main lignan in sesame seeds and oil is sesamin. It is highly hydrophobic and easily obtained as a crystalline product from the scum obtained by the vacuum-deodorization process in the purification of unroasted sesame oil production (Kushiro et al., 2002).

Sesamin and sesamolin lead to the formation of sesamol and sesaminol during refining or hydrogenation; sesamol disappears appreciably during deodorization, sesaminol is less affected by this stage of refining (Tashiro, 1990).

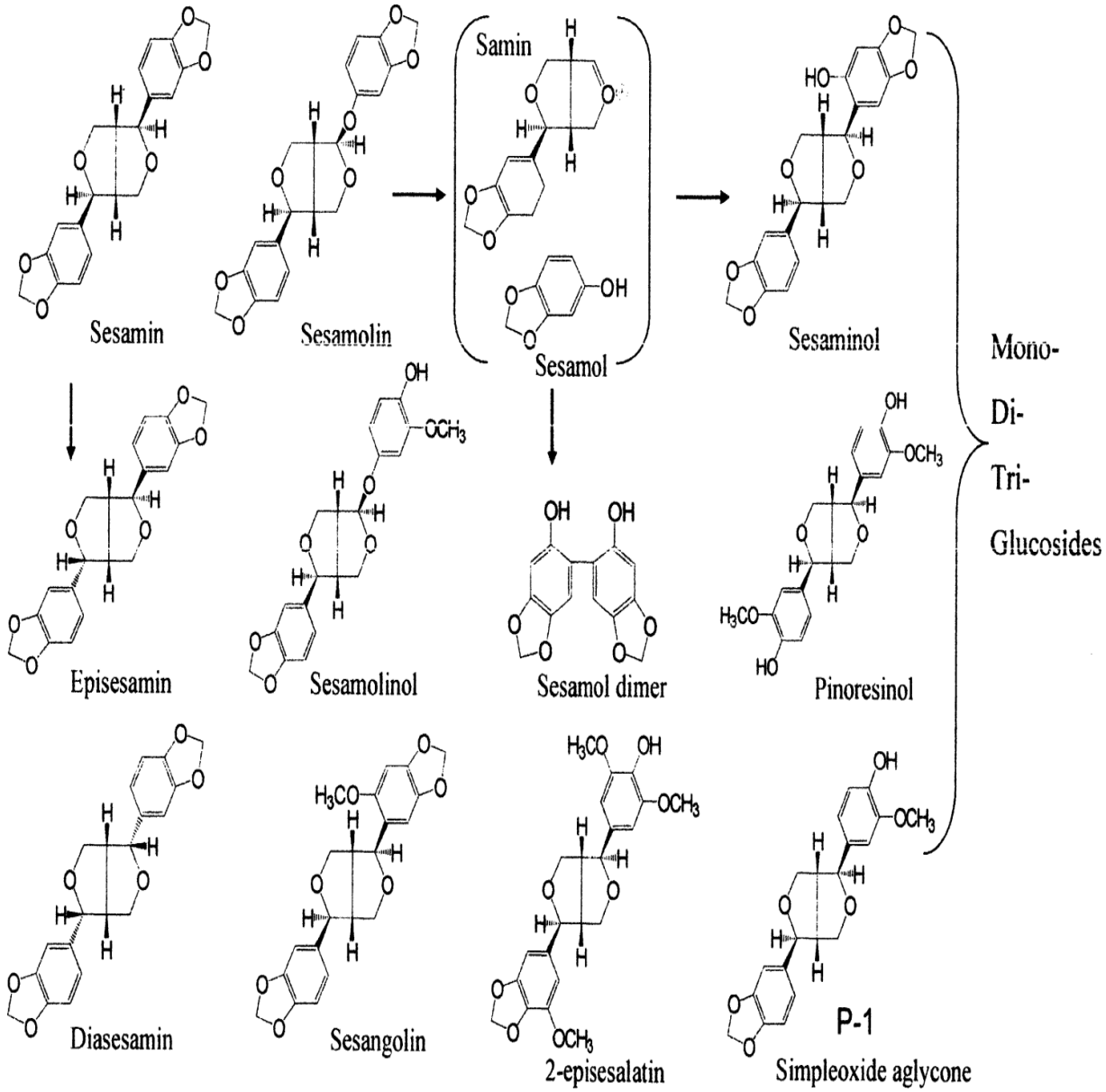


Figure 6: Sesame lignans and related compounds (Namiki, 2007)

II.3. Antioxidant potential of sesame oil

Sesame oil has long been recognized for its stability. In ancient Egypt, it was used in mummification because they considered it resistant to oxidative degradation (Namiki et al., 2007). Today, these beliefs are confirmed by scientific research in which several types of vegetable oils have been stored at 60 ° C in the free area. Sesame oil remained stable after 50 days while there was a rapid increase in oxidative degradation after 10 days for the other vegetable oils tested (Namiki et al., 2007; Fukuda et al., 1988).

II.4. pharmacological properties of sesame lignans

In 1990, a first study was made to verify the anti-aging effect of sesame using mice whose aging process is accelerated. A diet containing 20% sesame effectively slows aging, reduces lipid peroxidation and increases the activity of the superoxy-dedismutase (SOD) enzyme involved in free radical scavenging (Hunot et al., 1999).

Sesamin has gained much attention, precisely after the works of Hirata and his collaborators who have demonstrated for the first time its cholesterol-lowering effect on humans (Hirata et al 1996). Sesamine affects lipid metabolism by decreasing the absorption of cholesterol in the small intestine and decreases the activity of two enzymes involved in the synthesis of cholesterol, 3-hydroxy-3-methylglutaryl CoA reductase and Δ-5 desaturase (Gao et al., 2008) and also suppresses cholesterol buildup in the liver of rats and hamsters (Ogawa, et al., 1994). Sesamin has also been proven to decrease lipogenesis by decreasing lipogenic enzymes of liver. It has been shown to decrease the lipogenic gene expression of sterol regulatory element binding protein-1 (SREBP-1), acetyl-CoA carboxylase and fatty acid synthase, that means less fat is esterified in the liver and therefore less fat synthesis (Ide et al., 2003). The effect of sesamin on enzymes involved in catabolism and anabolism of fatty acids is summarized in table VII.

Table VII: Effect of sesamin on enzymes involved in catabolism and anabolism of fatty acids (Anilakumar et al., 2010).

Enzyme	**Nature of activity**
Catabolism of fatty acids (â-oxidation)	
Carnitine palmitoyltransferase	Activation
Acyl-CoA oxidase	Activation
3-hydroxyacyl-CoA dehydrogenase	Activation
3-ketoacyl-CoA thiolase	Activation
2,4-dienoyl-CoA reductase	Activation
Ä3, Ä2 - enoyl-CoA isomerase	Activation
Anabolism of fatty acids (lipogenic activity)	
Fatty acid synthase	Inhibition
ATP-citrate lyase	Inhibition
L-Pyruvate kinase	Inhibition
Glucose-6-phosphate dehydrogenase	Inhibition

Ketogenesis occurs when fatty acids oxidation is enhanced to a level that the liver cannot metabolize all them to produce energy. Excess acetyl-CoA, generated from fat, glucose and amino acid catabolism is converted to ketone bodies in the liver and released into the blood-stream for use by other tissues especially the brain. Ketogenesis is an important process during low carbohydrate diets because the brain uses only glucose as fuel. When glucose is low, the brain will turn towards ketone bodies for its energy. Sesamin has been shown to increase the production of ketone bodies. Increased production of ketone has protein-sparing effect as less amino acids are needed to create ketones eventually sparing muscle mass while dieting (Fukuda, 1999).

Sesamin also decreases the risk of arteriosclerosis in humans by lowering low density lipoprotein (LDL) which are responsible for transporting cholesterol to cells and it also regulates the omega 6 / omega 3 ratio to keep it in the normal values (Namiki, 2007).

Unlike estrogen, sesamin has the ability to inhibit the growth of human cancer cells, especially breast cancer. The growth of these cancer cells is stopped in the GI phase and is due to the increased degradation of cyclin DI by sesamin. Cyclin DI is a proto-oncogene which is overexpressed in several human cancer cells (Mogi et al., 1996).

Sesamolin (figure 6) has many important pharmacological properties: antioxidant, antibacterial and antiproliferative, cholesterol-lowering, anti-hypertensive and neuro-protective effect (Rangkadiloket al., 2010). A study by Cooney and his collaborators has shown that the consumption of moderate amounts of sesamolin significantly increases the blood level of γ-tocopherol, the latter is recognized to be effective against the proliferation of cancer cells in the prostate and breast (Cooney et al ., 2001).

Sesamol (Figure 6) which is a phenolic derivative molecule (produced during the decomposition of sesamolin at high temperature) with a methylene-dioxide group, is known to be a powerful antioxidant. Shenoy and these collaborators reported that sesamol is very effective for wound healing in albino rats (Shenoy et al., 2001).

I.2.4. Other antioxidants in sesame oil

Many studies have led to the identification of other metabolites and substances responsible for the antioxidant activity of sesame oil such as thiazole, pyrroles, cetones, aldehydes, phenolic acids, phytosterols (beta-sitosterol, stigmasterol, Campesterol, sigmasterol-3-O-β-D-glucoside), verbascoside, rhamnetine, Kaempferol-3-O-β-D-glucuronide and ferulic acid (Saha 2014, Botelho et al. , 2014; Dada and Adeparusi, 2012).

III. Probiotics and prebiotics

III.1. Probiotics

III.1.1.Definition

The notion of probiotics was developed mainly from the works of Metchnikoff, researcher at the Institut Pasteur and Nobel Prize in 1908 (Drider and Prévost., 2009). The term "probiotic" was first introduced in 1954 by Vergio who compared in his manuscript "Anti- und Probiotika" the effects of antibiotics and other antimicrobial substances on the intestinal flora (Holzapfel and Schillinger., 2002) . In 1965 Lilly and Stillwell defined them as microbiological factors capable of stimulating the growth of other organisms like those composing the intestinal flora. Parker (1974) then extends this definition to "organisms and substances which contribute to the balance of flora". This definition potentially includes microbial metabolic products including antibiotics (Kipnis et al., 2012).

Later, Roy Fuller in 1989 proposed a definition very close to the current meaning: "living microbial food supplement which beneficially affects the host by improving the balance of its intestinal flora" (Agrawal., 2005). In contrast to the previous definitions, the following definition introduces the notion of defined strain that is well characterized from a taxonomic point of view as well as the notion of quantity brought to humans. This is the universal definition established by the WHO / FAO: "living microorganisms which, when administered in adequate quantities, have a beneficial effect on the health of the host which ingests them" (Illupapalayamet al. , 2014; Tripathi and Giri., 2014).

III.1.2.The different types of probiotics

The microorganisms known to be probiotics are generally lactic acid bacteria belonging to the genus *Lactobacillus* and *Bifidobacterium* but certain bacteria belonging to other genera and some yeast and molds also have probiotic functions. (Rivera-Espinoza et Gallardo-Navarro, 2010 ; Kruger., 2009). The types of microorganisms known to be probiotics are illustrated in table VIII.

Tableau VIII **:** Microorganisms known to be probiotic (Tripathi and Giri., 2014; Saad *et al.*, 2013).

Lactobacillus	Bifidobacteria	Other lactic acid bacteria	Other microorganisms
L. acidophilus	*B. adolescentis*	*Enterococcus faecalis*	*Bacillus* spp,
L. amylovirus	*B. animalis*	*Enterococcus faecium*	*Escherichia coli* strain Nissle
L. brevis	*B. bifidum*	*Lactococcus lactis*	*Propionibacterium*
L. casei	*B. breve*	*Leuconstoc mesenteroides*	*freudenreichii*
L. cellobius	*B. infantis*	*Pediococcus acidilactici*	*Clostridium butyricum*
L. crispatus	*B. lactis*	*Sporolactobacillus inulinus*	*Saccharomyces cerevisae*
L. curvatus	*B. longum*	*Streptococcus thermophilus*	*Saccharomyces bourlardii*
L. delbrueckii	*B. thermophilum*	*Streptococcus diacetylactis*	
L. farciminis		*Streptococcus intermedius*	
L. fermentum		*Streptococcus salivarius*	

a) Lactic acid bacteria

Lactic acid bacteria are heterotrophs, the characteristics of which are as follows: gram-positive bacilli or shells, generally immobile, asporulated, aerotolerant, chemotrophic and do not have catalase, nitrate reductase and cytochrome oxidase (Drider and Prévost., 2009). They are classified according to two criteria: their morphology and their fermentation path (Rivera-Espinoza and Gallardo-Navarro, 2010). They represent 1% of the fecal flora of an adult (Buddington et al., 2002). They form an extremely important and interesting group of bacteria due to their numerous metabolic activities which contribute to the highly appreciated organoleptic characteristics of fermented products, in particular flavor, texture as well as nutritional and technological attributes (Nout, 2009). They have the capacity to degrade a wide range of oses such as lactose and galactose for dairy products, but also sucrose, maltose, glucose, fructose and -galactosides for products of plant origin (Goulet 2009).

b) Lactobacillus

Lactobacillus has the longest history of health benefits associated with microorganisms. It is this genus which was at the origin of the first probiotic theories stated by Metchnikoff

(Sharma and Mridula., 2013). This genus includes at least 145 species recognized for - unlike bifidobacteria - present a phylogenetic, phenotypic diversity and extremely varied and ecological. Lactobacillus rhamnosus GG is the most studied probiotic strain, around 200 references (AFSA, 2005). Lactobacillus acidophilus is the most dominant genus of the intestinal tract of healthy humans. It is resistant to digestive enzymes and bile salts and tolerates acidity up to a pH of 3.6 (ie 0.3% to 1.9% of triturable acidity); this is why it is considered probiotic (Rivera-Espinoza and Gallardo-Navarro, 2010).

c) Bifidobacterium

Bifidobacterium is a normal component of the gastrointestinal tract of humans and animals (shizuka et al., 2012). It's a gram positive non sporulated with a particular Y-shaped, immobile morphology, negative catalase and anaerobic (Chitapanarux et al., 2015; Martinez et al., 2013). Its optimum pH is between 6 and 7 and its growth is low at pH less than 4.4. Its optimum growth temperature is between 37 and 42 (Rivera-Espinoza and Gallardo-Navarro, 2010). This bacteria is predominantly prevalent in children who are breastfed (Agrawal., 2005). During an infectious episode such as diarrhea, the number of these bacteria decreases in the stool (Colarelli., 2010). The species Bifidobacterium animalis initially named Bifidobacterium bifidum before being reclassified is one of the probiotic strains that received the most attention; it is well characterized and has recently been sequenced, which facilitates its study (Leivers et al., 2011).

III.1.3. Marketing form of probiotics

Probiotics are consumed in two main forms: capsule supplements and food (Champagne and Gardner, 2005). Encapsulation (or microencapsulation), which is a new technology, makes it possible to increase the survival of probiotics by offering them maximum protection against physicochemical agents and macrophages (Martine et al., 2008). However, the benefits of probiotics in this form do not equal those of a probiotic food. In fact, the short time spent in the intestine could prove to be insufficient for the metabolic activation of lyophilized (encapsulated) strains (Ejtahed et al., 2011). In addition, food is the most popular form for the consumption of probiotics. For these foods, the quantity and variety of which are exploding on the markets, the challenge lies mainly in maintaining the survival of microorganisms (Saran et al., 2012).

III.1. 4. Survival of probiotics in digestive tract

The amount of live probiotics in the gut depends on the strain, the dose ingested, host factors and the carrier food. Many strains of bifidobacteria and lactobacilli survive well during intestinal transit and arrive in large quantities in the stool. Gastric acidity and biliopancreatic secretions are the main endogenous mechanisms for inactivation of ingested bacteria (AFSA. 2005). Protection against gastric acidity can be done by a rapid passage in the stomach or by protecting bacteria by the buffering capacity of the vector food or by galenical protection systems such as microencapsulation (Drider and Prévost., 2009).

The ingested dose of probiotics is an important factor in achieving the desired effects. It is recommended that the product should contain at least 10^7 CFU / ml of live probiotics at the time of consumption (Lamsal and Faubion, 2009). For example, this amount had to be consumed for *Lactobacillus rhamnosus* GG to be detected in the feces. It is also often cited that probiotic concentrations must be greater than or equal to 10^6 CFU / ml in the small intestine (ileum) and 10^8 CFU / g in the colon (AFSA. 2005).

III.1.5. Selection criteria of probiotics

Choosing a microorganism as a probiotic is tricky since it will have to demonstrate certain characteristics to be considered as such. The criteria most discussed in the literature are summarized in the following table:

Tableau IX : The most criteria used for probiotic selection (Tripathi et Giri., 2014).

Food safety and security	➢ Non-pathogenic strain and identified by appropriate methods characterized by phenotypic and genotypic techniques. ➢ No possible transmission of antibiotic resistance genes.
Technological criteria	➢ Stability during the production process by resistance to the manufacturing conditions of the product (temperature, pressure, oxygen). ➢ Survival in the food until it is consumed. ➢ Possess interesting organoleptic characteristics and should not alter the product.
Functional criteria	➢ Tolerance to stomach acid (pH as low as 1.5 on an empty stomach). ➢ Resistance to bile and digestive enzymes. ➢ Ability to adhere to epithelial cells and mucus. ➢ Confronting the indigenous microbiota by production of antimicrobial substances and antagonism towards pathogens (bacteriocins; H2O2). ➢ Resistance to phages, viruses that affect bacteria.
Desirable physiological criteria	➢ Production of essential vitamins and fatty acids ➢ Positive effect on the immune system and reduction of digestive inflammatory responses ➢ Reduction of diarrhea and constipation and metabolism of cholesterol. ➢ Reduced risk of colon cancer ➢ Reduction of symptoms due to lactose intolerance.

III.1.6. Benefit effect of probiotics

The most documented effects are on the digestive tract; the immune system and host metabolism (Piquet et al., 2007).

III.1.6.1. Effet on digestive tract

The digestive health benefits of probiotics can include:

• Reducing the incidence and the severity of diarrhea (childhood diarrhea, diarrhea associated with antibiotics, traveler's diarrhea), are the most universally recognized pathology benefits from the use of probiotics (Ducrotté., 2010; Agrawal ., 2005).

• Treatment of constipation, bloating and Irritable Bowel Syndrome by helping to soften the stool and make it easier to pass through the colon. Probiotics can promote regularity and control intestinal transit time, and can help increase the frequency of bowel movements (Waikar., 2013; Ducrotté., 2007).

• Prevention and treatment of pouchitis, which is an inflammation of the ileal reservoir in patients who have undergone colon removal (Drider and Prévost., 2009).

• Reduction of the risk of colon cancer in the animal model, probably because they reduce the activity of certain bacterial enzymes which could increase the level of procarcinogens (Goulet 2009).

• We speak of a real "barrier effect": the probiotic prevents the fixation of the pathogenic agent, by inhibiting its binding to receptors or adhesion molecules, consequently it reinforces the secretion of mucus from the intestinal cells in order to strengthen the barrier of the wall against attacks (Drider and Prévost., 2009). The mechanisms involved in the modulation of the barrier effect are illustrated in the following figure:

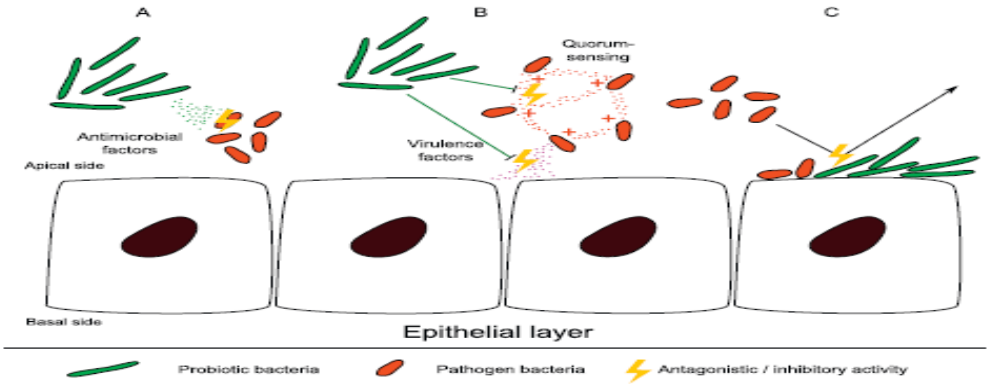


Figure 7**:** Modulation of the barrier effect. A. Antimicrobial compounds production. B. Quorum-sensing and virulence factors production inhibition. C. Cell adhesion competition (Alexandre *et al.*, 2014)

III.1.6.2. Effect on host metabolism

The most remarkable metabolic effects are:

• Promoting the solubilization of various constituents contained in food, thus improving their bioavailability. In this regard, yogurts have Ca, Mg and K levels around 30% higher than those of original milks (Ngounou et al., 2003). Improved absorption of these minerals is promoted by increased production of acids in the colon. This effect can positively influence the risk of osteoporosis and increases bone strength (Kruger., 2009; Delzenne and Cani. 2008)

• Reduction of the effects of lactose intolerance because the lactose digestibility of yogurt can be up to 90%. The lactase in yogurt is said to be protected from gastric acidity and released in the duodenum under the action of bile acids (Sharma and Mridula., 2013).

• Improvement of the digestibility of starch, due to the amylolytic activity of certain amylolytic lactic acid bacteria (BLA) (Ouattara et al., 2008)

• Provide group B vitamins, especially riboflavin, niacin and thiamin (Holzapfel and Schillinger, 2002).

• Metabolism of cholesterol and consequently reduction of its circulating rate in the blood (Rivera-Espinoza and Gallardo-Navarro, 2010).

• Reduction of anti-nutritional factors such as: α-galactosides (stachyosis and raffinose responsible for the phenomenon of flatulence), phytates, phenolic compounds which decrease the bioavailability of minerals (iron, zinc and calcium), in particular tannins protease inhibitors (trypsin inhibiting factor), (Lestienne et al., 2005).

III.1.6. 3. Effect on the immune system

The positive effects of probiotics on the immune system are currently attributed to the fact that they modulate the composition of the natural microflora in the intestine. These immuno-modulatory effects are varied:

- ☞ Activation of local macrophages and increased presentation of antigens to B lymphocytes (Moreau., 2001),
- ☞ Increased production and secretion of immunoglobulins A and decreased immune response to food antigens (Kipnis et al., 2012).

- ☞ Production of anti-inflammatory cytokines (IL-10, TGF-B) (Ribeiro et al., 2011)
- ☞ Reduction of intestinal permeability (Goulet 2009)

One of the criteria frequently used to select probiotic strains is their ability to inhibit pathogenic bacteria. The most used method is co-incubation on an agar or liquid medium allowing the growth of the probiotic and of the pathogenic agent most often belonging to the genera Bacillus, Clostridium, Enterococcus, Escherischia coli, Listeria, Pseudomonas, Salmonella, Staphylococcus and Yersinia (Dunne et al., 2001). Probiotics can inhibit the development of these pathogenic bacteria by producing antibacterial factors such as hydrogen peroxide (H_2O_2) and Nitric oxide free radical (NO), by clumping them together or by competition for nutrients (Rivera-Espinoza and Gallardo-Navarro, 2010).

Several lactic acid bacteria can also effectively detoxify the mycotoxins contained in plant-based foods, generally synthesized by molds such as Aspergillus, Byssochamys or Penicillium. Mycotoxins are recognized as mutagens, carcinogens, and immunosuppressants. Patulin (PAT) and ochratoxin (OTA) are degraded by some lactic acid bacteria (Speijers, 2003).

III.2. Prebiotics

III.2.1. Definition

The prebiotic concept has been linked to the development of functional foods since the 1990s (Dupont., 2000). Gibson and Roberfroid (1995) gave the first definition of this new category of food, the characteristic of which is to be "non-digestible substances which induce a beneficial physiological effect on the host by specifically stimulating growth" and / or the activity of a limited number of bacterial populations already established in the colon such as bifidobacteria and lactobacilli"

Thus, the principle of prebiotics is based on the selective stimulation of these colonic microorganisms capable of degrading these prebiotics into monomers of carbohydrates which they use for their growth (Kruger 2009) so that a food ingredient is classified as prebiotic, it must both:

1. Resist stomach acid and enzymatic hydrolysis and not be absorbed in the upper part of the digestive tract;
2. Be a selective fermentation substrate for one or more beneficial, commensal colon bacteria;

3. Selectively stimulate the growth and / or activity of intestinal microorganisms which provide beneficial health effects, consequently inducing a healthier composition of the colonic flora (Gibson et al., 2004);
4. Be stable during the processing of the food that contains it (Wang et al., 2009).

A probiotic can be associated with a specific substrate, belonging to the class of prebiotics. The mixture thus formed is then called a symbiotic: a fructo-oligosaccharide can be associated in this way with a strain of bifidobacteria or else lactitol with a lactobacillus (Rodrigues et al 201; Gibson and Roberfroid, 1995).

III.2.2. Nature of prebiotics

Prebiotics are carbohydrates and specifically oligosaccharides or polysaccharides including some dietary fibers (Lamsal and Faubion., 2009; Gibson et al., 2004). We distinguish fructo-oligosaccharides, galacto-oligosaccharides, arabino-galactooligosaccharides, gluco-oligosaccharides and inulin (Boual et al., 2011). Many other carbohydrates can integrate the group of prebiotics such as xylo-oligosaccharides, isomalto-oligosaccharides, manno-oligosaccharides, soy oligosaccharides, pectic oligosaccharides, sugar alcohols, lactulose, even lactose in the deficit lactase (Wang et al., 2009; Zeanandin et al., 2011). Resistant starch is also considered a prebiotic (Sharma et al., 2008). Some examples of prebiotics on the market are illustrated in the following table:

Table X: Examples of prebiotic compounds marketed (Grizard and Barthomeuf, 1999; Franck, 2002).

Prebiotic	Name	Structure
Oligofructoses	Raftilose®	$Fru\text{-}Fru_n + Glc\text{-}Fru_n$
Fructo-oligosaccharids	Actilight®	$Glc\text{-}Fru_n$
Galactooligosaccharids	Oligomate®	$Glc\text{-}Gal_n$
Lactulose	MLS-50®	Gal-Fru
Soya oligosaccharids	Soya-Oligo	$Gal_n\text{-}Glc\text{-}Fru$
Isomaltooligosaccharids	IMO 900	Glc_n
Glucooligosaccharids	Bioecolia®	Glc_n
Mannooligosaccharids	Bio-MOS®	Man_n
Xylooligosaccharides	Xylo-oligo	Xyl_n

- **Galacto-oligosaccharids (GOS)**

They are produced by trans-glycosylation of lactose by β-galactosidase preventing yeasts, molds and bacteria (Figure 18). The result is an oligosaccharide complex with different degrees of polymerization (DP). The DP varies between 2 and 8 and the possible connections are of type β (1–1), β (1–2), β (1–3), β (1–4) and β (1–6) (Figure 8).

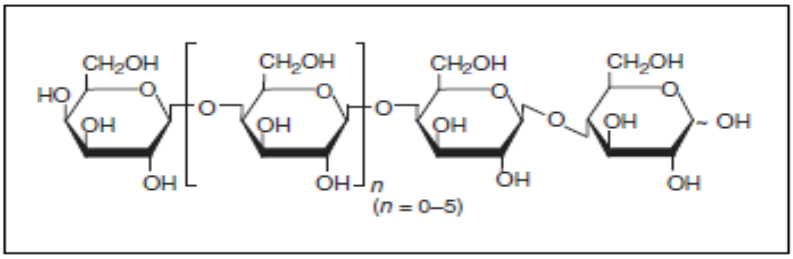


Figure 8**:** Structure of galacto-oligosaccharides (GOS) of type-β 1,4 (Sako *et al*., 2011).

Due to this variation in bond types, several isomers exist. This β bond is hydrolyzed in the colon by bifidobacteria and lactobacilli. The diversity in the types of bond and in the degree

of polymerization takes into account that bacteria have preferences for a few molecules for their fermentation. Indeed it has been proven that the strain Bifidobacterium longum subsp. infantis ATCC 15697 prefers GOS with a DP = 4. However, the strain Bifidobacterium adolescentis ATCC 15703 preferably uses GOS with a DP = 3 (Ladirat., 2014).

- **Inulin**

It is a fructose polymer that is found in its natural state. It is not hydrolyzed by human digestive enzymes but by the ß-fructosidase of bifidobacteria (Aryana and McGrew., 2007). The fructosyl radicals are united with each other with β (1-2) bonds (figure 9). Inulin is found in chicory roots, artichokes, asparagus, Jerusalem artichokes, onions, garlic, leeks, bananas ... (Dacosta, 2001).

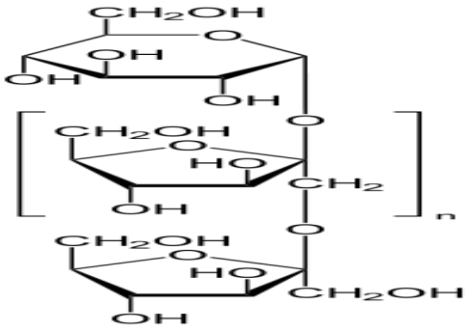


Figure 9: Structure of inulin (Sako *et al*., 2011).

- **Fructo-oligosaccharides (FOS)**

FOS Actilight and FOS Raftilose belong to the fructan family. Those are variable length fructose polymers which can be derived from simple fructose polymers or fructose elements attached to a sucrose molecule by β- (2 → 1) bond. They exist naturally in a number of plants including onion, chicory, artichoke, garlic, Jerusalem artichoke and endive (Grizard and Barthomeuf., 1999).

III.2.3. Beneficial effects of prebiotics

Experimental data show that the administration of prebiotics of the fructan, arabinoxylan, glucan type, characterized by their bacterial fermentation in vivo, makes it possible to reduce adiposity, steatosis, glycemia, and endotoxemia in many persons. The administration of prebiotics reveals an almost systematic increase in the number of bifidobacteria, which appear

as the "bacterial signature" of their effect on the microbiota (Delzenne et al. 2012). The results of some studies show the beneficial effects of prebiotics are summarized in the following table:

Tableau XI : Somes positives affect of prebiotics

Prébiotic	**Effets bénéfiques**	**Références**
FOS (Raftilose)	➢ Increase in total fecal mass, ➢ Improved absorption of minerals such as Ca, Mg, Fe and Zn	(Delzenne *et al.*, 1995)
Gluco-oligosaccharide (GOS α-1,6)/ trans-oligosaccharides (TOS)	➢ Increase in the number of bifidobacteria ➢ Colonic pH reduction	(Bouhnik *et al.* 1998)
FOS (Actilight)	➢ Increased number of bifidobacteria in the colon and stool	(Qing *et al.*, 2003)
Arabino-xylooligosaccharide (AXOS, Opti'Flor®).	➢ improve metabolic parameters; dyslipidemia and metabolic abnormalities in an experimental mouse model suffering from CKD (chronic renal failure), ➢ Reduction in plasma urea level ➢ Decrease in total plasma cholesterol and triglycerides ➢ Reduction of sugar level in blood	(Buddington *et al.*, 2002)

III.2. Sesame carbohydrates

Sesame seeds are a rich source of carbohydrates, which represent between 20 to 25% of the dry weight of the seed (Namiki, 2007). At present, no studies have been conducted on the prebiotic effect of carbohydrates from sesame.

White sesame processed, unprocessed, and dehulled seeds have been analyzed for the total carbohydrate composition. They contained glucose (3.63%), galactose (0.40%), fructose

(3.43%), sucrose (0.17%), raffinose (0.59%), stachyose (0.38%), planteose (0.23%), and sesamose (0.14%).

Penta- and hexa-saccharides (0.12 and 0.04%, respectively) obtained were shown to be the higher homologues of the planteose series. Sesame seeds contain hemicellulose A and hemicellulose B in 0.58-2.34 and 2.71-2.59% yields, respectively. Hemicellulose A was found to be an acidic glucan containing galacturonic acid and glucose in a ratio of 1:12.9, while hemicellulose B contained galacturonic acid, glucose, arabinose, and xylose with trace galactose in a ratio of 1:3.8:3.8:3.1, respectively (Dharmaraj and Rudrapatnam 1976).

Experimental part

Chapter I: Effect of roasting on biochemical composition, quality and oxidative stability of sesame seeds and oil

I.1. Material and methods

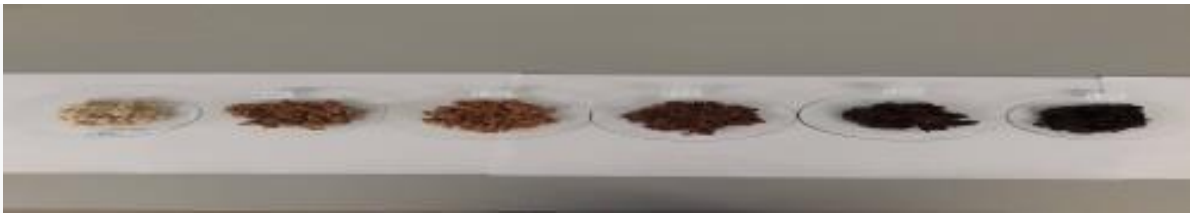
I.1.1. Plant material

White sesame seeds (*Sesamum indicum*) imported from India (figure 10) were purchased from a local market in Bejaia (Algeria). Impurities such as dust, sand, stones, spoiled seeds and other extra materials were separated using sieves. To study the effect of roasting on the composition and quality of the seeds and oil, sesame seeds were divided into two portions, one serves as raw sample and the other was roasted.

Figure 10: Photography of sesame seeds used in this study

I.1.2. Roasting and grinding

20 g of seeds are placed in a Petrie dish (8 cm in diameter) then introduced into an electric oven (Jeong et al., 2004) and roasted for 20 minutes at different temperatures (120, 150, 180, 210, 250 and 300 ° C). After cooling, the seeds are ground using an electric mill (GM 200; Retsch GmbH, Germany) to obtain a fine powder. The whole *Sesamum indicum* seed powders obtained are then stored in hermetically sealed containers, protected from light at 4 ° C to avoid any risk of oxidation or deterioration (figure 11).



A **B** **C** **D** **E** **F**

Figure 11: Photography of ground sesame seeds : A, unroasted; B, roasted at 150°C;C, roasted at 180°C; D, roasted at 210°C; E, roasted at 250°C,5F, roasted at 300°C.

I.1.3. Chemical composition of raw and roasted seeds

The total fat (TF) of the powders of roasted and unroasted seeds of *sesamum indicum*, are extracted with Soxhlet according to the of AOAC (1998) methods. Moisture was determined using AOAC (1998) method. Crude cellulose is the organic residue obtained after two successive hydrolyses (sulfuric acid then alkaline with soda) according to the method of Weende (Henneberg and Stohmann, 1860). The nitrogenous materials were determined by the Kjeldahl method (amount of nitrogen * 6.25). The content of mineral matter (ash) is determined according to the AOAC method (1990) by incineration of 1 g of sesame powder in a muffle furnace at 550 ° C for 5 hours. The determination of total sugars is carried out by the method of Dubois et al (1956). The latter requires acid hydrolysis which allows the breakdown of all carbohydrate bonds in the polysaccharide.

I.1.4. lipid extraction and characterization

Total lipids or fat were extracted with light petroleum ether (b.p. 40–60°C) in a Soxhlet extractor for 6 h (AOAC, 1998). After extraction, the solvent was evaporated under vacuum using a rotary evaporator Model Bibby Scientific (ST15 0SA, UK) and any residual solvent was removed by a gentle nitrogen steam. The final weight was used to calculate the oil content on a mass basis. The fat content is determined on 30 g of sample and 250 mL of solvent. The lipid content (expressed as% fat) is determined from the following relationship:

$$\mathbf{TL}\ (\%) = \frac{\mathbf{P2 - P1}}{\mathbf{P0}} \times \mathbf{100}$$

P2: represents the mass in grams of the balloon containing the fat; P1: represents the mass in grams of the empty balloon; P0: represents the mass in grams of the test portion.

The total fat of raw and roasted sesame seeds at different temperatures obtained by Soxhlet extraction was the subject of a physico-chemical characterization. The effect of culinary processing (roasting) on nutritional quality, oxidative stability and minor oil compounds has been established. The various physico-chemical tests which define the quality of the oils are carried out on all the samples.

I.1.4. 1. Physical and chemical properties

I.1.4. 1. 1. Density

The density of an oil is the ratio of the mass of a certain volume of this oil at 20 ° C, and the mass of an equal volume of distilled water at the same temperature (AOAC, 1998; Lion, 1955). To determine the relative density, a well-cleaned pycnometer is weighed empty (m_0), then weighed with 0.5 ml of distilled water (m_1) and finally weighed with 0.5 ml of oil sample (m_2). Pycnometers containing the sample and distilled water are placed in a water bath at a temperature of 20 ° C for 20 minutes (AOAC, 1998).

The density is determined using the following equation:

$$\mathbf{d20} = \frac{\mathbf{m2 - m0}}{\mathbf{m1 - m0}} \times \mathbf{100}$$

m0: mass in grams of the empty pycnometer, m1 : mass in grams of the pycnometer filled with distilled water, m2 : mass in grams of the pycnometer containing the oil.

I.1.4. 1. 2. Refractive index

The refractive index "RI" of a substance is the ratio between the speed of light (of determined wavelength) in air and the speed of this same light in this substance (Adrian et al., 1998 ; Laisney, 1992). This parameter is used for identification and as a criterion for the purity of oils.

The refractive index is measured according to the official AOAC method, (1998). 10 g of oil are first heated in a water bath (40 ° C). A few drops are deposited between the prisms of the refractometer so as to completely fill the space between these prisms. After a few minutes, allowing the fat to reach thermal equilibrium, the value given by the refractometer (figure 12) is noted.

Figure 12: Refractometer (Abbe 1T/ 4T)

I.1.4. 1. 3. Color determination

Color is an important attribute of the quality of oils and provides information on the intensity of heat treatment. The color parameters are determined using a Konica Minolta type colorimeter (Chroma Meter CR-400, Japan).

The color parameters L *, a * and b * represent the clarity, the green-red color and the blue-yellow color respectively.

L *: represents the clarity where L * = 0 (black), L * = 100 (white).

a *: represents the green-red color, from green (-a *) to red (+ a *).

b *: represents the blue-yellow color, from blue (-b *) to yellow (+ b *).

The effect of seed roasting on the color of extracted oils obtained is achieved with a Konica Minolta device, calibrated on a white background. A plastic cup with a diameter of 3 cm is filled with the oil to be analyzed, to take the readings. The color measurement is carried out in the dark and the values of the different color parameters (L, a, b) are given directly by the colorimeter at the same time.

I.1.4. 2. Chemical properties of oils

I.1.4. 2. 1. Acid value

The acid value is the amount in milligrams of potassium hydroxide (KOH) necessary to neutralize the free fatty acids contained in 1 g of fat (Ogbunugafor et al., 2011).

2.5 g of sample are dissolved in 50 mL of the diethylic ether / ethanol mixture (95: 5, v / v) previously neutralized. The titration is then carried out with a potassium hydroxide solution (0.1 mol / L) in the presence of phenolphthalein as a colored indicator until a persistent light pink color is obtained (AOAC, 1998). The acid value is then determined using the following relationship and the acidity is expressed as a percentage of oleic acid:

$$\mathbf{A} = \frac{\mathbf{N} \times \mathbf{V} \times \mathbf{Eqg}}{\mathbf{m}}$$

N: represents the normality of potassium hydroxide, V: volume of KOH for the sample in mL, m : mass of sample in grams. Eqg : represents the gram equivalent of KOH= 56,1 Eqg/mol.

I.1.4. 2. 2. Iodine value

This parameter allows the measurement of unsaturation degree of fat by determining the number of iodine in grams which fixes it on the double bonds present in 100g of lipids. It is determined using the Wijs reagent and with titration with a sodium thiosulfate solution in the presence of starch as a color indicator (Sudke and Sakarkar, 2013).

In a Erlenmeyer flask, 0.3g of the oil is mixed with 25ml of Wijs reagent. 20ml of potassium iodide (10%) and 150ml of distilled water are added to this mixture and the whole is well shaken and then incubated for 1 hour in the dark. Titration with the 0.1N sodium thiosulfate solution (Na2S2O3) is carried out in the presence of a few drops of starch paste as an indicator until the black blue color disappears (Sudke and Sakarkar, 2013)

The iodine value is given by the following formula:

$$\mathbf{IV} = \frac{\mathbf{N} \times (\mathbf{V} - \mathbf{V0}) \times \mathbf{12,69}}{\mathbf{m}}$$

With,

N is Normality of the solution (0,1N),

V_0 : Volume of $Na_2S_2O_3$ (ml) necessary to titrate the blank test,

V : Volume of $Na_2S_2O_3$ (ml) necessary to titrate the sample,

m : weight of sample (g).

I.1.4. 2. 3. Saponification value

The saponification index is the amount of potassium hydroxide (in milligrams) required to saponify 1 g of fat. This value is higher since the fatty acids are of low molecular weight. Its principle remains on a reflux boiling of a test portion with a potassium hydroxide solution, followed by a titration of the excess potassium hydroxide (KOH) with a hydrochloric acid (HCl) solution (AOAC, 1998).

2 g of oil are dissolved in 2 ml of the potassium hydroxide solution. After heating the solution under reflux for one hour, 0.5 to 1 ml of the phenolphthalein solution are immediately added to the mixture and then titrated with a hydrochloric acid solution (0.5 N) until the pink color completely disappears (AOAC, 1998).

The saponification value is calculated by the formula established below:

$$\mathbf{SV} = \frac{\mathbf{N\ x\ 56,1\ x\ (Vo - V)}}{\mathbf{m}}$$

Vo: volume in ml of HCl used for the blank test; V: volume in ml of HCl used for the sample to be analyzed; m: test sample in grams; N: normality of hydrochloric acid HCl 0.5N.

I.1.4. 3. Oxidative stability of oils

I.1.4.3.1. Absorbance in the ultraviolet or specific extinctions

Absorbance in the ultraviolet (UV) is a parameter which provides information on the quality of an oil, on its freshness and its storage conditions (ISO 3656, 2002). The oxidation of a fatty substance leads to the formation of "hydroperoxides" which absorb light at 232 nm. If oxidation continues, secondary oxidation products are formed, in particular "unsaturated ketones" which absorb light at 268 nm (Tanouti et al., 2010).

The specific extinctions at 232 nm and 268 nm are determined according to the EEC method N.2568 / 91. One drop of oil is dissolved in 10 mL of isooctane; after homogenization with a vortex, the absorbance's are measured using isooctane as white using a spectrophotometer (Spectrostar nano, BMG Labtech GmbH, Germany).

The specific extinction at 232 nm and 268 nm are calculated using the formula Next :

$$£_{1cm}^{1\%} = A(\lambda)/w$$

A (λ): absorbance at the wavelength λ, w: the concentration, in g / 100 mL, of the sample in solution, K232: specific extinction at λ = 232 nm, K268: specific extinction at λ = 268 nm.

I.1.4.3.2. *Para*-anisidine value (*p*-anisidine)

The *para*-anisidine (PV) value allows the measurement of secondary oxidation compounds generated following the decomposition of hydroperoxides. This is a parameter that indicates the presence of "unsaturated aldehydes" in fats and oils (Casal et al., 2010). The measurement of unsaturated aldehydes is determined according to ISO 6885.2 (2006) method.

0.5 g of oil is dissolved in 10 mL of isooctane to prepare a solution test (ST) and two solutions are prepared from it: A solution called "solution before reaction" by adding 1 mL of

glacial acetic acid to 5 mL of the ST. A solution called "solution after reaction" by adding 1 mL of the *p*-anisidine solution prepared in acetic acid to 5 mL of the ST. A third solution (white) is prepared by adding 1 ml of the solution of *p*-anisidine to acetic acid to 5 ml of isooctane. Solutions are left in the dark for 10 minutes to produce a complex colored, and the absorbance is measured at 350 nm using a spectrophotometer (Shimadzu UV 1800 spectrophotometer, Japan). The *p*-anisidine value is calculated as follows:

$$\mathbf{PV} = \frac{\mathbf{100\ QV}}{\mathbf{m}}\mathbf{1,2(A1 - A2 - A0)}$$

PV: the p-anisidine value, V: volume in which the sample is dissolved in mL, m: mass of sample in g, Q: sample content of the solution measured in g / mL (Q = 0.01g / mL), A0: absorbance of the solution before reaction, A1: absorbance of the solution after reaction, A2: absorbance of the blank, 1.2: correction factor for the dilution of 5 mL of the test solution with 1 mL of the reagent.

I.1.4.3.3. Rancimat (accelerated oxidation)

This test assesses the resistance capacity of a fatty substance under the extreme conditions of aging and oxidation, which are high temperature and oxygen.

This method is based on the measurement of the induction period (IP, hours), which corresponds to the time when the formation of oxidation products begins to increase rapidly. It is determined according to the ISO 6886 method: (2006), by measuring the oxidation induction time, using a professional Rancimat 982 device (Metrohm, Switzerland).

This device works by contacting an air flow (20 L / h) with 3 g of sample of oil heated to 120 ± 1.5 ° C; the volatile compounds are collected in water and the increase in the conductivity of this water is continuously measured. The time required to reach the inflection in conductivity is recorded. Oxidative stability is estimated during induction time and given in hours. A higher IP correspond to more resistant to oxidation.

I.1.4. 4. Determination of polar compounds

Estimation of total and individual polar compounds was carried out using previously described methods (Márquez-Ruiz et al.,, 1996).

I.1.4. 4. 1. Solid-phase extraction (SPE)

A solid-phase extraction was used to separate polar and non-polar fraction from oil samples. 1 mg of internal standard IS (10 mg/mL of monostearine in THF) was measured and the solvent THF was evaporated, then 50 mg of oil was added and completed to 2 ml with petroleum ether then shaken vigorously. The silica columns for SPE were conditioned before use by rinsing with 10 mL of initial solvent hexane-diethyl ether 87:13; (v/v) to avoid columns from drying out. 2 mL of sample solution (containing oil and IS) were placed on the column and the solvent (10 mL) of hexane-diethyl ether 87:13; (v/v) was passed through to elute non-polar fraction of the sample.

Next the polar fraction of sample was eluted with 10 ml of diethylic ether. The two fractions were evaporated in nitrogen steam then redissolved in 1ml of tetrahydrofuran for next analyses by high performance size-exclusion chromatography.

I.1.4. 4. 2. High performance size exclusion chromatography (HPSEC)

The fractions of polar compounds recuperated from SPE were analyzed by HPSEC in a JASCO chromatograph (Jasco, Japan) with a Phenomenex column (Phenogel, 100A°, 600 mm × 7.8 mm ID, 5µm film) and refraction index detector. The mobile phase used was THF with a flux of 1mL/min. The injected volume was 20 µL in ambient temperature.

Quantity of total polar compounds (PC% on sample) was determined using the following equation:

$$\mathbf{PC\%} = \frac{(\mathbf{\Sigma A} - \mathbf{Ais}) * \mathbf{Cis}}{\mathbf{Ais} * \mathbf{Coil}} * \mathbf{100}$$

Where, ΣA is the sum of areas of all peaks, Ais is the area of internal standard peak, Cis is the internal standard concentration in the initial samples solution (mg/ml) and Coil is the oil concentration in the initial sample solution (mg/mL).

The relative amounts of each type of compounds, including triglyceride polymers, triglyceride dimmers, oxidized triglyceride monomers, diglycerides and fatty acids, were calculated from the individual peak areas and percentage of total polar compounds, assuming equal response factors.

I.1.6. Fatty acid composition and profile

I.1.6. 1. Preparation of methylic esters

FA composition of roasted and unroasted sesame oil was determined by gas-liquid chromatography with flame ionization detection (GC-FID). Before analysis, fatty acids were subjected to cool alkaline trans-esterification to produce fatty acid methyl esters (FAMEs) as detailed in Regulation EEC 2568/91 (Regulation, 1991).A solution of 40 mg of oil dissolved in 2mL of heptane and 50 µL of internal standard solution was shacked with Na_2SO_4 and 0.2 mL of 2N methanolic potassium hydroxide. To stop esterification, $NaHSO_4$ was added then the mixture was centrifuged at 3000 tr/min during 10 minutes.

I.1.6.2. Fatty acid profile determination with GC

FAMEs and standards were analyzed using a Chrompack CP 9001 gas chromatograph (Chrompack, Middelburg, Netherlands).The injector type was a programmed temperature vaporizer, operating in split/splitless mode. The gas chromatograph was equipped with a flame ionization detector, an auto sampler (Chompack CP-9050) and a 50 m×0.25 mm id fused silica capillary column coated with a 0.19 µm film thickness (FAME Select, JW, Varian).The split ratio was 1:50 and the injected volume was 1.2 µL. The carrier gas used is helium with an internal pressure of 120 kPa. Temperature setting was as follows: detector 250, and injector 230°C.

Identification of the fatty acid methyl esters (FAME) was based on the comparison of their retention times with those of FAME standards. The quantification was made by using peak areas in electronic units and the fatty acid content was expressed in relative percentage of each fatty acid. In addition, the fatty acid isomers: C18:1_T9, C18:2_T9T12, C18:2_C9T12; C18:2_T9C12 were identified with individual standards.

I.1.7. Determination of minor compounds (unsaponifiable matter) and antioxidant potential of oils

I.1.7.1. Determination of lignanes and tocopherols

Accurate portions (≈50 mg) were weighed in to Eppendorf tubes and IS (1 µg) was added. Samples were dissolved in n-hexane up to 1 mL, and anhydrous sodium sulfate was added (around 100 mg). After centrifugation (2 min.; 13,000 rpm), the supernatant was analyzed immediately.

Analyses were carried out according to the method developed previously (Cruz & Casal, 2013) using an integrated system with a data transmitter (Jasco LC – NetII/ADC, Japan), pump (Jasco PU-980, Japan), a refrigerated auto-sampler (Jasco AS – 2057 Plus, Japan) and a fluorescence detector (Jasco FP-2020 Plus, Japan) programmed for excitation at 290 nm and emission at 330 nm, gain 10. Data were analyzed using Chrom NAV Control centre – JASCO Chromatography Data Station.

The chromatographic separation was achieved with a normal phase column (Luna Silica; 100 mm × 3 mm; 3 µm) (Phenomenex, USA). The isocratic system comprised a mobile phase mixture of 1,4-dioxane in n-hexane (3.5%, v/v) at a flow rate of 1 mL.min-1, operating at constant room temperature (22 ± 2 ºC), and the injection volume was 20 µL.

The compounds were identified by chromatographic comparisons with authentic standards for the tocotrienol, or with fractionated compounds from oil (sesamin and sesamolin) in accordance with (Takahashi et al., 2016) and by their UV spectra comparison, using a PDA detector (Jasco MD-2015 Plus, Japan). Quantification was based on the fluorescence signal response, using the internal standard method, with the standards calibrated on the basis of their chromatographic purity, and published extinction coefficients (Bhatnagar et al., 2015; Nesaretnam, et al. 2007)

I.1.7.2. Sterols composition and profile by GC-FID

The Sterol profile of both unroasted and roasted sesame oils was determined according to the European Union Regulation 1348, 2013 (Regulation 1348, 2013) using gas chromatography equipped with flame ionization detection.

First the sterol fraction was separated from the unsaponifiable matter. 50 µL of internal standard α-cholestanol (0.2%) was put in a vial then evaporated in nitrogen. Into the same flask, it was added 0.1 g of oil and 5 mL of methanolic KOH 2M, then the mixture was heated at 80°C during 40 minutes. 5 ml of water was added and wait for cooling to room temperature. Unsaponifiable phase was extracted with 6 mL of diethyl ether and the organic phase was transferred (top) to a vial of 40 ml. The extraction was repeated twice.

The organic fraction was washed with 5 ml H_2O and this procedure was repeated until the water stop producing a pinkish color by adding one drop of phenolphthalein. The water residue was removed using anhydrous $Na_2SO_4.5H_2O$. After centrifuging, the extract was transferred into a vial of 15 ml capacity and evaporated to 1 ml. The solution was transferred

to 2 ml vial and evaporated the solvent in nitrogen steam. The residue was dried in oven at 100°C for 15 min and allows cooling. The betulin standard (20 ul) was added then evaporated, after 500 ul of acetone was added and evaporated again to dryness. The residue obtained corresponds to the sterol fraction and triterpenic diols. 100 µl of the derivatization reagent was added and shaken until complete solubilisation then heated at 80°C for 15 min (oven) then centrifuged 3 min at 4000 rpm. Pass the derivatized to a new insert.

This fraction was next analyzed in gas chromatography FID (TRACE GC; Thermo Finnigan, Italy), with a 30 m × 0.25 mm i.d., 0.25 µm DB-5MS column (Agilent J&W Scientific, Folsom, CA).

The initial temperature of the column was 250 °C and increase at a rate of 2 °C/min to 280 °C, with a helium flow of 1.0 mL/minute and internal pressure of 100 kPa. The injection was made with an automatic injector (Thermo Scientific Al 1310, Italy) with split ratio of 1:10 and injected volume of 1.5 µL. Each sterol in the chromatograms was identified by comparing retention time of standards and quantified by computer control using area normalization. The concentration of each individual sterol, in mg/kg of oil was calculated as follows:

$$\textbf{Sterol X} = \textbf{Ax} * \textbf{ms} * \frac{\textbf{100}}{\textbf{As}} * \textbf{m}$$

Where:

Ax : is the peak area for sterol x, in computing system counts;

As: is the area of the α-cholestanol peak, in computing system counts;

ms: is mass of added α-cholestanol in milligrams;

m :is masse of sample in grams.

- The percentage of each individual sterol is calculated as following:

$$\textbf{Sterol X} = \frac{\textbf{Ax}}{\sum \textbf{A}} * \textbf{100}$$

Where Ax is peak area for x and ΣA is total peak area for sterols.

I.1.7.3. Determination of carotenoids

Quantification of total carotenoids (TC) present in roasted and unroasted sesame oils is achieved using the method described by Nagata and Yamashita, (1992). 0.3 g of oil sample is

dissolved in 10 mL of an acetone / hexane mixture (4: 6, v / v). The solution is well homogenized with a vortex. The absorbance readings of the samples are taken at wavelengths 663nm, 645 nm, 505 nm and 453 nm using a spectrophotometer (Spectrostar nano, BMG Labtech GmbH, Germany). The determination in total carotenoids is expressed in mg / kg of oil.

The total carotenoid content is calculated using the following relationship:

$$TC=0,216xA_{663}–1,22xA_{645}–0,304xA_{505}+0,452xA453$$

TC: Total carotenoids in mg / 100g oil; A663: absorbance at 663 nm; A645: absorbance at 645 nm; A505: absorbance at 505 nm; A453: absorbance at 453 nm.

I.1.7.4. Determination of total phenolics

Total phenolic content (TPC) was determined in the oils using the Folin–Ciocalteu reagent. First 50 mg of oil in Eppendorf was diluted until 2 mL with ethyl acetate. In a Falcon of 10 mL it was added 100 uL of oil sample solutions and 500 µL of Folin-Ciocalteu reagent freshly prepared. The mixture was homogenized and incubated 5 minutes at room temperature in the dark. 3 mL of Na_2CO_3 (10 g/100 mL) were added and, vortexes during 30 seconds, then the mixture was adjusted with deionized water until to 10 mL, vortexed, and left for 90 minutes at room temperature in the dark. The obtained solution was centrifuged for 5 minutes at 5000 r.p.m then the absorbance was read at 765 nm with a blank containing 100 µL of water.

A calibration curve was performed with gallic acid and the results of total phenolics were expressed in mg gallic acid equivalent/100g of oil.

I.1.7.5. Determination of antioxidant activity by DPPH test

The antioxidant activity of the roasted and unroasted oils was evaluated by assessing their ability to scavenge the 2,2'-diphenyl-1-picrylhydrazyl radical. To 500 µL of sample (oil diluted in ethyl acetate) were added 3.5 mL of DPPH agent (2.5 mg diluted in 100 mL ethyl acetate). The mixture was homogenized, incubated 30 min at room temperature in the dark, centrifuged during 5 min at 5000 r.p.m, and then the absorbance was read at 517 nm. Calibration curve was made using gallic acid and the results of antioxidant activity were expressed in mg gallic acid equivalent/100g of oil.

I.1.8. Statistical analysis

All experiments were carried out in triplicate and the values are expressed in mean ± standard deviation. Results were analyzed for variance (ONE-WAY ANOVA), followed by a multiple comparison of means (LSD test) using the software Statistica version 7.1. The differences are significant at $P<0.05$.

I.2. Results and discussion

In this part, the results of different analyzed parameters were reported and discussed. This section was interested to evaluate the effect of seed roasting on biochemical composition, quality and oxidative stability of sesame seed and oil.

The symbols used for different samples were given as following:

USS: Unroasted Sesame Seeds; **RSS**: Roasted Sesame Seeds;

USSO : Unroasted Sesame Seeds Oil; **RSSO**: Roasted Sesame Seeds Oil;

I.2.1. Overall biochemical composition of roasted and unroasted sesame seeds

Biochemical composition of roasted and unroasted sesame seeds are detailed in figure 13. Significant reductions in the levels of proteins (3.6%), moisture (25.6%), cellulose (4.0%) and ash (24.5%) with roasting were observed. But in the case of fat and ash it was observed a significant enhancement at 250°C and 180°C respectively.

Heat is known to improve the availability of some nutrients, inactivating enzymes that accelerate the spoilage of food nutrients. It also destroys microorganisms and positively changes the physical appearance and attributes of food such as color, texture and flavor.

Roasting is a major step in the processing of seeds and nuts. In general, heat pre-treatment improve sensory quality, and shelf life of food products. Roasting facilitates oil extraction by destroying cell barriers, and degrades allergenic substances in nuts and seeds. Oily seed roasting is practiced in the oil industry in many areas of the world, and can affect the properties of the seed and oil for further applications. Maillard reaction products were reported to lengthen the induction period of oil oxidation and decrease the rate of oxidation at the propagation step (Elizalde et al., 1991).

The yield in crude oil before roasting was 48.84%. This value was within the range reported for *S. indicum* oil from different origins: Pakistan that varied between 47.5 and 53.9% (Asghar & Majeed, 2013), Morocco with 52% (Gharby et al., 2017).

RSSO samples showed significantly higher ($p < 0.001$) oil yields than the USSO one. An increase in roasting temperature results in an increase in extraction yield with its maximum (63.12%) at 250°C. Alasalvar et al. (2010) also found that roasting enhanced oil level in Turkish hazelnut (*Corylus avellana* L) varieties.

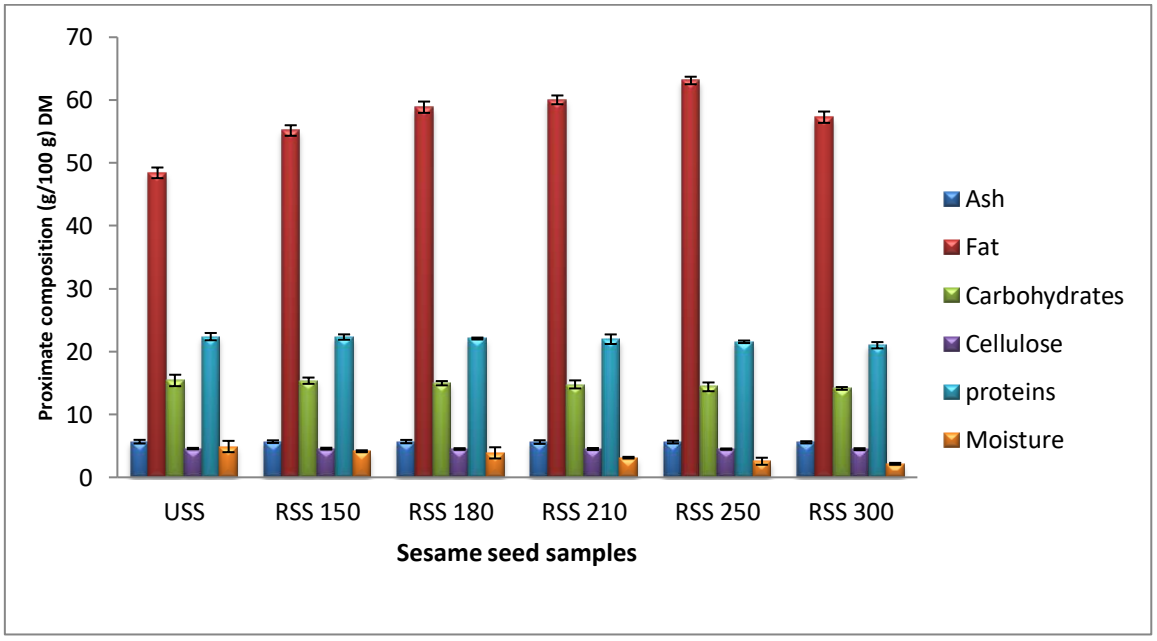


Figure 13: Proximate composition of row and roasted sesame seeds

Roasting sesame seeds at different conditions was found to increase oil yields with increasing temperature and time (Yoshida & Takagi, 1997). This is probably due to the damage caused in seeds cell membranes by protein denaturation during heating (Yoshida & Kajimoto, 1994). So, the cells released their content leading to improves oil extractability (Lee et al., 2004).

During food processing, cooking and roasting, the application of dry heat has mixed effects on its nutritional value. Roasting of corn and rice leaded to the release of niacin from niacytine, while cooking the corn caused the release of free nicotinic acid. Nutrient loss is one of the undesirable changes that occur in food processing (Ayatse et al., 1983; Bassir & Lawal, 1985).

I.2.2. Physical proprieties of extracted oils

I.2.2. 1. Moisture of sesame oils

Figure 14 illustrates moisture results of sesame seeds oils. The moisture content in food is a critical factor that affects their conservation, because the humidity can be the origin of numerous chemical reactions of degradation and microbiological contaminations. This parameter was highly affected by roasting temperature ($P < 0.001$). Moisture of oil before roasting was 2.55% then it was reduced until 0.15% at 300°C, among 94% of water content was eliminated under this high temperature.

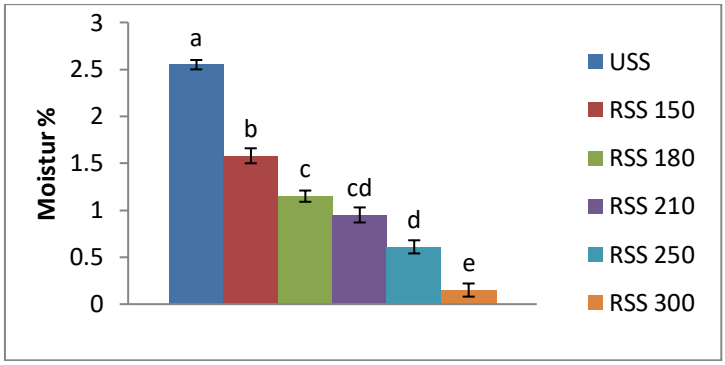


Figure 14: Moisture percentages of sesame seeds oils. Values followed by different letters are significantly different ($P < 0.05$).

I.2.2. 2. Density (specific gravity) and refractive index

The results of density and refractive index of the oils of *Sesamum indicum* unroasted and roasted at different temperatures are shown in Figure 15. Refractive index is mainly used to characterize changes in unsaturation as the oil is hydrogenated; it increases with increasing in level of double bounds (Dim, 2013).

Refractive index for USSO was 1.476 which is near to values reported for sesame from different origins (Borchani et al., 2010; Dim, 2013; El Khier et al., 2008). For all the oils, refractive index varied between 1.461 to 1.476, it decreases by increasing roasting temperature.

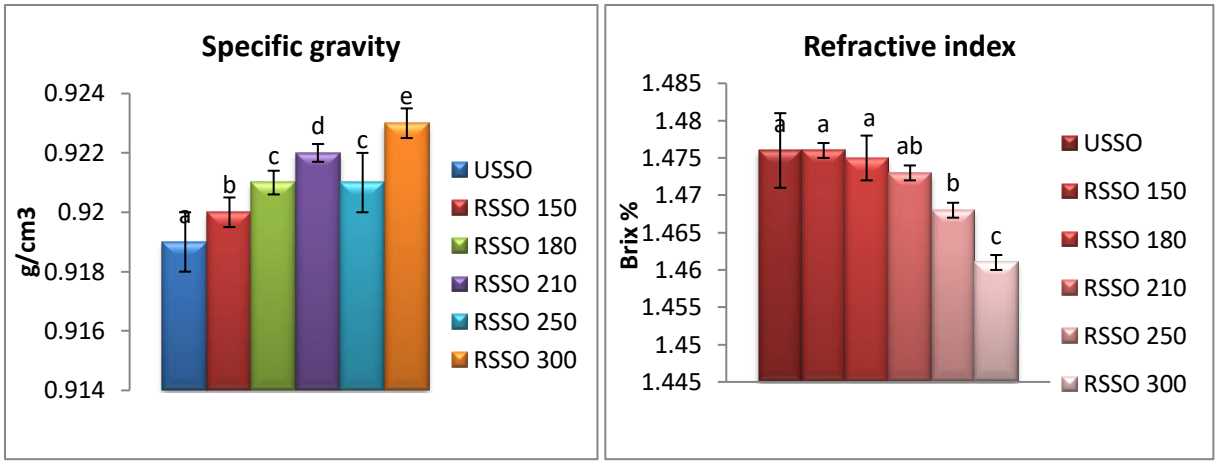


Figure 15: Density (specific gravity) and refractive index of sesame seeds oils. Values followed by different letters are significantly different ($P < 0.05$).

Specific gravity for USSO was 0.919, which is in agreement with that previously found by many authors (Dim, 2013; El Khier et al., 2008; Mohammed & Hamza, 2008). Globally after roasting, refractive index was affected ($p < 0.05$) whereas specific gravity was not ($p > 0.05$).

I.2.2. 3. Color

Visual color of seeds and oils is an important attribute that provides information on the intensity of heat treatment. The color development of sesame oils is illustrated in figure 17. It changed gradually from light yellow in USSO to brown (under 210°C) and finally to deep-brown (at 300 °C) as shown in the figure 16.

Globally seeds oils color was highly affected ($p<0.0001$) by roasting temperature. Highest Lightness (L) value was recorded in USSO (48.66) and decreased with increasing temperature.

Figure 16**:** Color evolution of unroasted and roasted sesame seed oils.

We can see (figure 17) an important linear increase in red color (*a*) between 210°C and 300°C (R^2=0.97) that resulting from browning substances like melanoidin formed by non-

enzymic Maillard reaction (Baisier & Labuza, 1992) between reducing sugars from glycolipids and free amino acids or amid molecules (Koechler & Odell, 1970).

Phospholipids degradation was also reported to cause browning during roasting process (Husain et al., 1986). Similar results were observed with sesame (Ji et al., 2019), wheat germ (Zou et al., 2018), rice germ, cashew (Chandrasekara & Shahidi, 2011a) and sunflower oil (Lee et al., 2004; Yoshida et al., 2002).

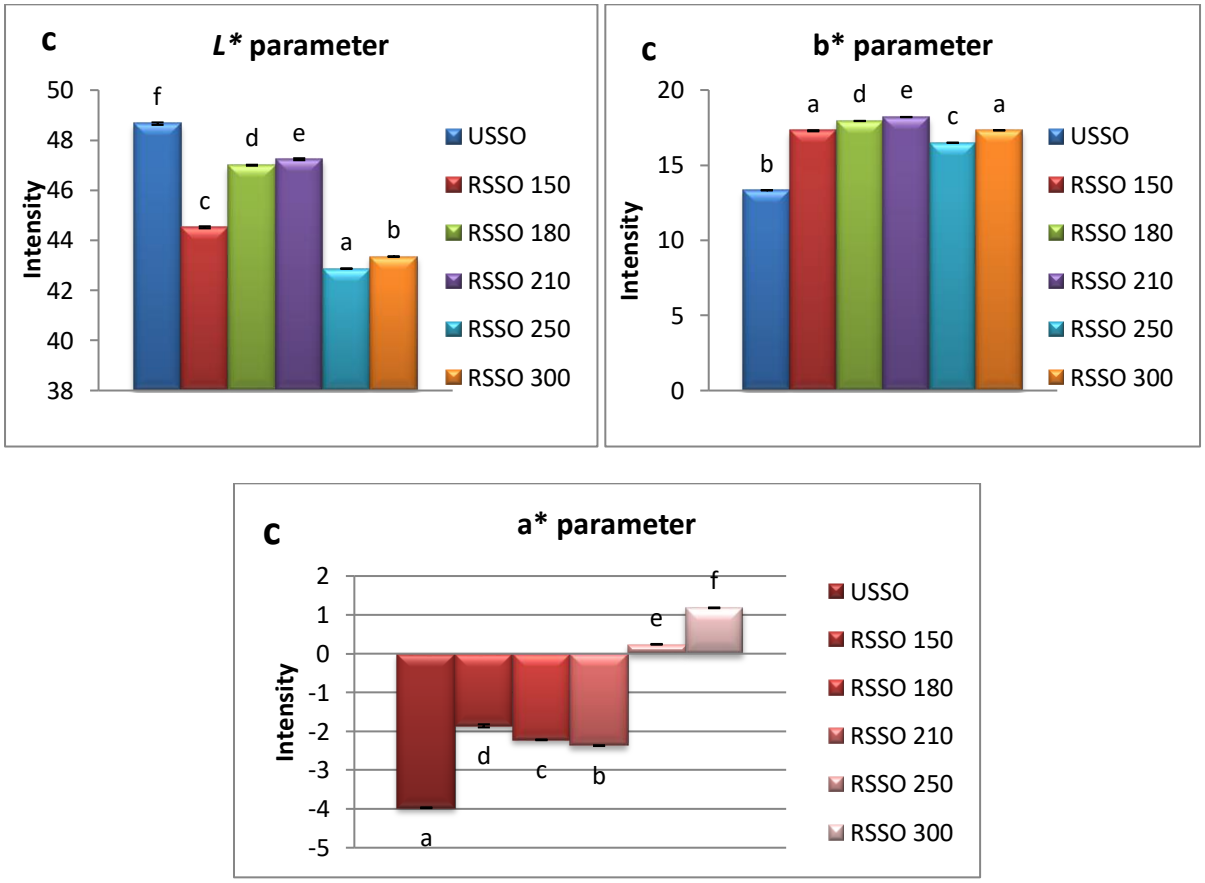


Figure 17**:** Color parameters of sesame seed oils. Values followed by different letters are significantly different ($P < 0.05$).

I.2.3. chemical properties of extracted oils

I.2.3.1. Acid value

This parameter provides information on the level of free fatty acids contained in a fat and makes it possible to judge its state of deterioration. The release of fatty acids is due to the enzyme activity in the oil samples. Statistical analysis of the results reveals a significant effect of the temperature factor ($P < 0.05$). The acidity of *Sesamum indicum* oil was 2.91%, it has

been reduced to 2.52% after roasting the seeds at 150 ° C (figure 18). On the other hand, an increase in acidity is observed from the temperature of 250 ° C.

The elimination of water can be at the origin of the reduction of acidity due to the inhibition of enzymes; and the increase in acidity at high temperatures is due to the effect of heat which releases fatty acids from triglycerides.

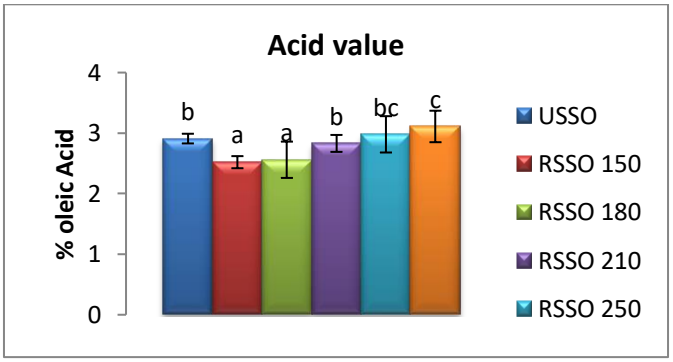


Figure 18: Acid value of sesame oils. Values followed by different letters are significantly different ($P < 0.05$).

I.2.3.2. Iodine value

Iodine value (IV) is generally used to measure degree of unsaturation in fatty acids of triacylglycerols (Dim, 2013; Gharby et al., 2017), solidification temperature and oxidation stability (Hrastar et al., 2012). High values indicates that oil contains greater number of double bonds (Zine et al., 2013).

The iodine value of USSO was 113.11 gI_2/100 g oil which decline until 103.17 mg/100 g in oil extracted from seeds roasted at 300°C (figure 19). The same result was reported in pumpkin seed oil roasted at different temperatures (90 to 200°C) but with a very fast decrease in IV, from 155 to 79 and from 146 to 75 g I_2/100g oil, for Gleisdorf and Rustikal respectively (Potočnik et al., 2018).

The protective roles of sesame lignans as antioxidant agents resulted in a smaller decrease in double bonds (Konsoula & Liakopoulou-Kyriakides, 2010).The decrease in IV after roasting is due to the double bonds destruction by oxidation, polymerization or scission favorized by high temperature (Hassan, 2012).

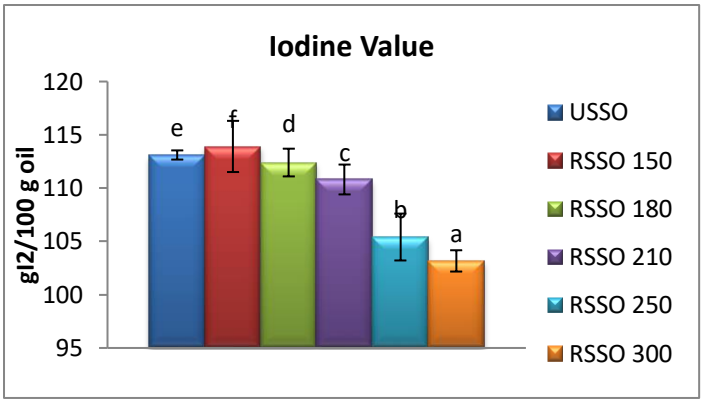


Figure 19: Iodine value of sesame oils. Values followed by different letters are significantly different ($P < 0.05$).

For both IV and refractive index, there were no great differences when roasting temperatures were under 250°C. However refractive index and iodine value decreased suddenly when the roasting temperature was above 250°C, resulting from reducing in saturation level.

I.2.3.3. Saponification value

The results of saponification value (SV) are detailed in figure 20. SV is an indicator of average molecular weight (it has an inverse relationship with molecular weight) that gives information on the alkali-reactive groups in oils.

The SV obtained before roasting was 185.45 g KOH/100 g oil, it increased until 200.05 g KOH/100 g when the roasting temperature was above 300°C. This result was in agreement with that reported by Yen (1990) wherein increase of SV from 183.5 to 199.2 at 260°C was observed (Yen, 1990). A saponification value of 200 mg KOH/g indicates high proportion of fatty acids of low molecular weight (Ezeagu et al.,, 1998); this can be explained by a decomposition of fatty acids at high temperature.

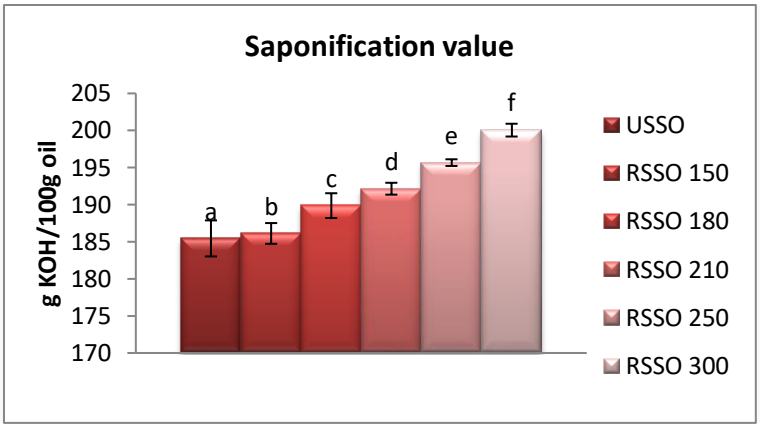


Figure 20: Saponification value of sesame oils. Values followed by different letters are significantly different ($P < 0.05$).

I.2.4. Oxidative stability of oils

The oxidative stability of seed oils before and after roasting was evaluated by their specific absorption, *p*-anisidine value and Rancimat method.

I.2.4.1. Specific extinctions

Specific extinctions or specific absorption at 232 nm (K232) and 270 nm (K270) indicates the production of conjugated dienes from PUFA (Mohamed & Awatif, 1998) and from primary and secondary oxidation products like carbonyl compounds and conjugated trienes (Casal et al., 2010). The results of the two parameters are illustrated in the figure 21.

The increase in roasting temperature yielded a significant ($p<0.001$) increase of both K232 and K270, showing that there was an increase of primary and secondary oxidation products. When the temperature was above 250°C, the increase of absorption was more important. This confirms the results obtained with IV and RI previously discussed.

A high and positive correlation was found between K 232, K 268 and the roasting temperature $y=0.009x+2.66$. $R^2= 0.76$; $y=0.002x+0.48$. $R^2=0.73$, respectively. Increase in lipid oxidation parameters that we have used in this study (*p*-anisidine, K232 and K270) revealed a progressive increase in primary and secondary lipid oxidation.

Based on the previous parameters we can deduce that from 250°C, there was an important lipid oxidation that can lead to the production of poor-quality oil.

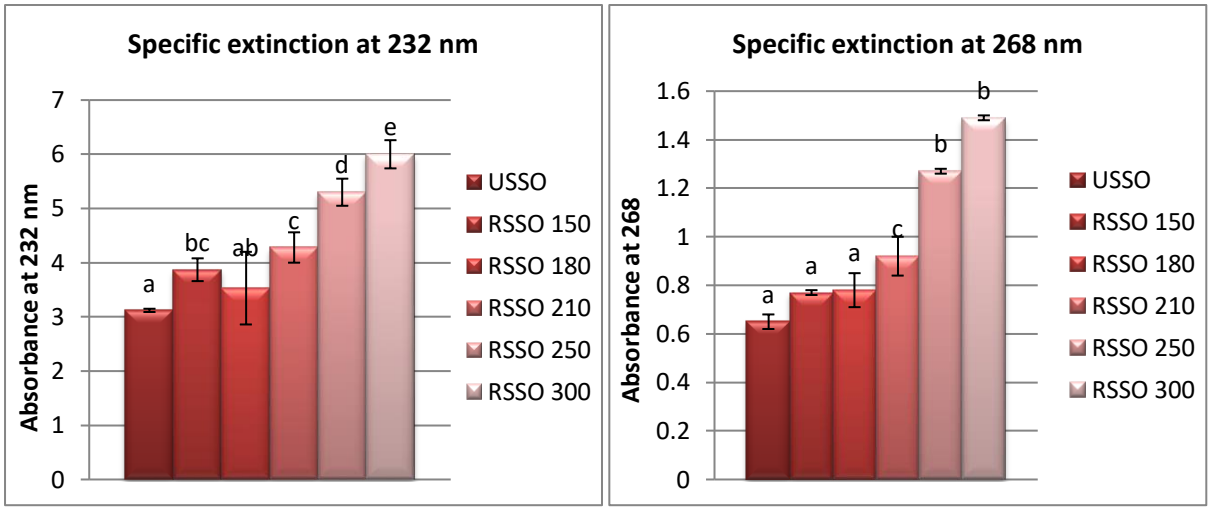


Figure 21: Specific extinctions of sesame seeds oils. Values followed by different letters are significantly different ($P < 0.05$).

I.2.4.2. Para anisidine value

p-anisidine value (figure 22) is characteristic of secondary peroxidation or unsaturated aldehydes production. Its value in USSO was within the range of reported values (Yaacoub et al., 2008; Yoshida & Kajimoto, 1994). At 300°C, it increased until 11.81 (12 times higher than that of unroasted seeds). This value was lower than that previously reported for sesame oils obtained from seeds roasted at 250°C for 30 min in domestic electric oven (Yoshida & Kajimoto, 1994).

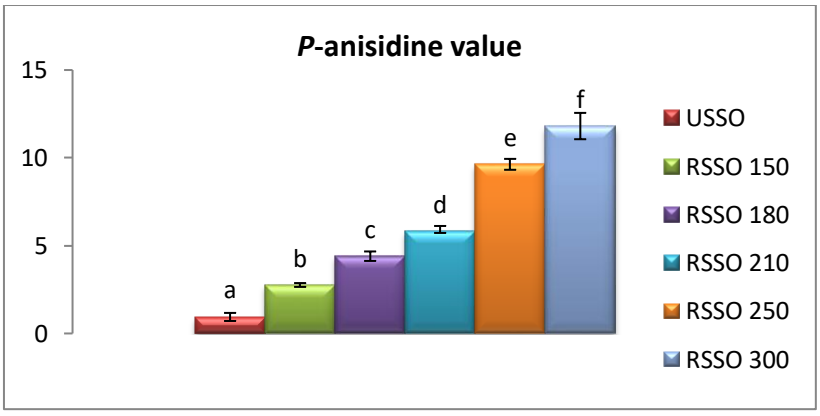


Figure 22: Para anisidine value of sesame seeds oils. Values followed by different letters are significantly different ($P < 0.05$).

The increase of this parameter is due to the important degradation of hydroperoxides primary products by lipid oxidation during heating leading to release of carbonyl compounds (Yoshida, Shigezaki, Takagi, & Kajimoto, 1995). The increase of *p*-anisidine was linearly proportional to the temperature increase ($y=22.83x+47.03$, $R^2=0.83$). The same correlation ($R^2=0.98$) was found between *p*-anisidine value and the temperature after roasting at 30 min (Yaacoub et al., 2009).

I.2.4. 3. Rancimat

The induction period (IP) of Rancimat method expressed in hours (h) is commonly used to evaluate the potency of oils and fats to resist (under stress conditions like air and heat) against accelerated oxidation (Chen & Ho, 1995).

The IP was significantly affected ($p<0.001$) by roasting temperature (figure 23), ranging from 5.51 to 10.5 h with a maximum in RSSO at 300°C and a minimum in USSO. Effectively, many authors have reported that stability of RSSO was more pronounced than that of USSO (Mohamed & Awatif, 1998; Yoshida et al., 2000; Yoshida & Takagi, 1997).This can be due to the newly formed compounds during roasting process. Indeed, it was reported that some lignans including sesamin and sesamolin , do not have antioxidative activity in themselves because of a lack of phenolic groups but sesamol which is present in trace in unroasted oil (released from sesamolin during heating) is considered as a strong antioxidant lignan. It was shown that γ-tocopherol from raw commercial sesame oil can't act as antioxidant agent (Fukuda, Osawa, & Namiki, 1981).

In 1986, Fukuda and his collaborators have shown a synergistic action between sesamol released from sesamolin and γ-tocopherol using a model system in linoleic acid. Addition of sesamol at 0.005% enhanced antioxidant potential of γ-tocopherol at all concentrations (Fukuda et al., 1986). Maillard reaction products has also been considered as antioxidant of processed foods (Amarowicz, 2009; Dewanto et al., 2002). Indeed, a research team has observed a small but synergistic effect between melanoidin (extracted from RSSO) and both of sesamol and γ-tocopherol using the thiocianate method (Yamaguchi, 1969).

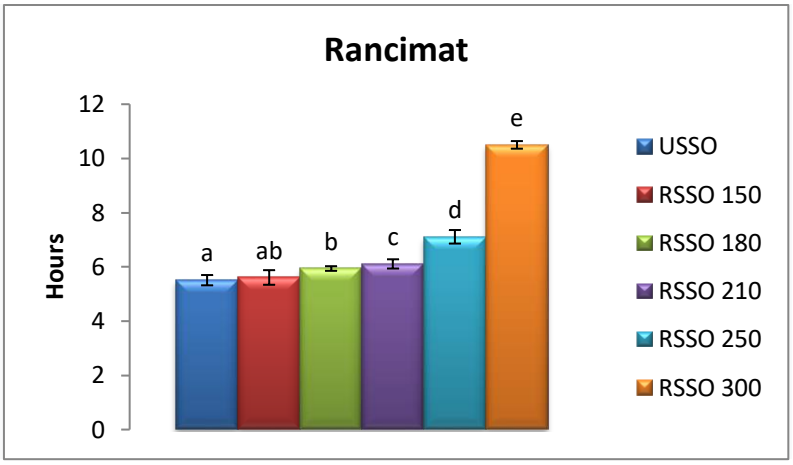


Figure 23: Rancimat test of sesame seeds oils. Values followed by different letters are significantly different ($P < 0.05$).

I.2.5. Polar compounds of sesame seeds oils

Quantification of polar compounds is one of the most used methods to evaluate the quality of edible oils especially those used in frying (Márquez-Ruiz et al., 1996). So it will be interesting to assess the total amount of newly formed compounds, with high polarity, in the seeds roasted at high temperature. Results are summarized in Table XII. Total polar compounds expressed in g/100 g of oil ranged from 5.7 to 6.7. The lower value corresponds to USSO while the higher one was exhibited by the RSSO at 300°C. All oils are under the limits established in different European countries for fat rejection (maximum 25%).

Table XII : Distribution of polar compounds expressed in relative % and total amount (g/100g oil) in oils.

	Samples					
Compounds	USSO	RSSO 150	RSSO 180	RSSO 210	RSSO 250	RSSO 300
TGP+TGD	0.1±0.00 b	6.7±0.9 c	4.7±0.64 a	5.2±097 a	5.4±0.54 a	9.5±0.01 d
oxTGM	18.8±0.67 c	22.5±0.03 ab	25.6±0.02d	22.1±0.06 a	23.7±0.13 b	22.9±0.21ab
DG	37.4±0.01 a	34.7±0.001 b	34.2±0.46 b	38.3±0.51 a	38.4±0.72 a	38.2±0.83 a
FFA	38.1±0.71 b	27±0.84 a	27.4±0.16 a	29.1±2.03 a	27.1±1.52 a	24.3±0.13 a
Total polar compounds (g/100g oil)	5.7±0.4 c	6.4±0.5 ab	6.3±0.3 ab	6.3±0.8 ab	6.5±0.1 a	6.7±0.01 b

Results are means ± standard deviations of the measurements. Values in the same colon followed by the same letter, are not significantly different ($p>0.05$). Abbreviations: TGP, triglyceride polymers; TGD, triglyceride dimmers; oxTGM, oxidized triglyceride monomers; DG, diglycerides; FFA, free fatty acids.

As expected, unroasted oil is characterized by a very low (0.1%) level of triglyceride polymers and triglyceride dimmers that are formed during soxhlet extraction. For others, it varied from 6.7 to 9.5%. Roasting at 150°C and 300°C had enhanced TGP + TGD by 95.52 and 98.94 %, respectively.

Globally, all the investigated oils presented a lower quantity of the maximum tolerated quantity in triglyceride polymers according to the European regulation (<14%), meaning that they are healthy products.

I.2.6. Fatty acid profile

Analysis by gas liquid chromatography revealed various fatty acids (FA) (Table XIII). FA composition of row sesame seed oil was similar to or within the range previously reported. (Biglar et al., 2012; Citil, Tulukcu, & Kocak, 2011; Mohamed & Awatif, 1998). The major FA recorded both before and after thermal processing were: linoleic (ranging from 43.62 to 44.39%),~oleic (ranging from 37 to 37.55%), palmitic (ranging from 9.21 to 9.38%) and stearic acid (ranging from 6.06 to 6.17%). The presence of high amounts of linoleic acid indicates that raw and roasted sesame are benefit and highly nutritious.

USSO and RSSO contained l6.24 to 16.50% saturated fatty acids (SFA), 38.25 to 38.94% monounsaturated, and more than 44% polyunsaturated fatty acids (PUFA). Statistically, there was a significant difference ($p<0.05$) between USSO and RSSO in the profile of FA. This result was in agreement with that reported for sesame (Yaacoub, Saliba, Nsouli, Khalaf, & Birlouez-Aragon, 2008) and almond kernels oils (Lin et al., 2016), whereas, several authors reported that oils from seeds prepared under different roasting temperatures and time remained unchanged (Ji et al., 2019; Yen, 1990; Yoshida, 1994).

As it can be seen in Table 3, the levels of SFA (C22:0, C16:0, C18:0) were increased while that of the unsaturated one (C18:1, C18:2, C18:3, C20:1) were declined. The change was slight and the difference is not statistically significant ($p>0.05$): decrease of 0.74 % in C18:1; 1.73 % in C18:2, 5.2% in C18:3 and 5.5% in C20:1. Several previous studies had investigated changes in FA and provide useful background. It was shown that roasting of brown sesame seeds have increased SFA; decreased linoleic acid from 46.1% to 24.8% and decreased α-linolenic acid from 0.4 to 0.2% (Hama, 2017). Contents of oleic and linoleic acids were drastically reduced when roasted at > 240°C for 30 min and the retention in total FA contents of oils prepared by roasting at 240 and 260°C for 30 min was 77.9 and 67.7%,

respectively (Yen, 1990). Roasting white and brown sesame seeds (from Far- North Region of Cameroon) at 180 °C for 10 min mostly affected PUFA for all sesame varieties (Tenyang et al., 2017). In kulthi seeds (*Dolichos biflorus*), the level of SFA slightly increased whereas that of unsaturated one decreased when roasting was carried out at 180°C for 20 min (Mishra et al., 2011).

Table XIII. Fatty acids composition (%) of unroasted and roasted sesame oils at different temperatures.

	Samples					
Fatty acid	**USSO**	**RSSO 150**	**RSSO 180**	**RSSO 210**	**RSSO 250**	**RSSO 300**
C14:0	0.02±0.00 a	0.02±0.00 a	0.02±0.00 a	0.02±0.00 a	0.02±0.00 a	0.02±0.00 a
C16:0	9.21±0.08 c	9.28±0.04 ab	9.22±0.01 a	9.3±0.00 ab	9.37±0.03bd	9.38±0.02 d
C17:0	0.07±0.00 b	0.06±0.00 ab	0.06±0.00 a	0.06±0.00 ab	0.06±0.00 ab	0.06±0.00 a
C17:1	0.02±0.00 a	0.02±0.00 a	0.02±0.00 a	0.02±0.00 a	0.02±0.00 a	0.02±0.00 a
C18:0	6.06±0.04 b	6.17±0.00 a	6.16±0.00 a	6.14±0.01 a	6.14±0.02 a	6.15±0.03 a
C18:1_T9	0.01±0.00 b	0.08±0.00 a	0.08±0.00 a	0.16±0.00 c	0.22±0.00 d	0.29±0.00 e
C18:1_C9	37.55±0.17 c	37.16±0.01 b	37.05±0.02 ab	37.13±0.02 ab	37.07±0.08 ab	37.00±0.03 a
C18:1_C11	0.93±0.01 b	0.69±0.00 a	0.91±0.01 b	0.92±0.00 b	0.91±0.00 b	0.92±0.00 b
C18:2_T9T12	0.00±0.00 a	0.00±0.00 a	0.00±0.00 a	0.00±0.00 a	0.01±0.00 b	0.02±0.00 c
C18:2_C912T	0.05±0.00 b	0.09±0.00 a	0.09±0.00 a	0.16±0.00 c	0.22±0.00 d	0.29±0.00 e
C18:2_T9C12	0.00±0.00 b	0.03±0.00 a	0.04±0.01 a	0.11±0.01 c	0.17±0.00 d	0.24±0.01 e
C18:2_C9C12	44.12±0.2 ab	44.35±0.03bc	44.39±0.01c	44.13±0.03 a	43.84±0.07 e	43.62±0.03 d
C18: 3_N3	0.8±0.00 a	0.38±0.00 a	0.38±0.00 a	0.37±0.00 ab	0.36±0.01 b	0.36±0.00 b
C20:0	0.66±0.01 a	0.66±0.01 ab	0.68±0.03 b	0.66±0.00 ab	0.66±0.00 ab	0.66±0.00 ab
C20:1C9	0.18±0.00 a	0.17±0.00 a	0.18±0.00 a	0.17±0.00 ab	0.17±0.00 b	0.17±0.00 ab
C21:0	0.01±0.00 a	0.01±0.00 a	0.01±0.00 a	0.00±0.00 a	0.00±0.00 a	0.01±0.00 a
C22:0	0.13±0.00 a	0.14±0.00 ab	0.14±0.00bc	0.14±0.00 ab	0.14±0.00 c	0.14±0.00abc
C24:0	0.09±0.00 a	0.06±0.00 a	0.09±0.00 a	0.09±0.00 a	0.08±0.00 a	0.09±0.00 a
C24:1	0.1±0.00 b	0.03±0.00 a	0.02±0.00 a	0.04±0.00 a	0.04±0.00 a	0.04±0.00 a
SFA	16.24±0.12 b	16.40±0.03 a	16.37±0.04 a	16.41±0.04 a	16.47±0.03 a	16.50±0.03 a
MUFA	38.94±0.14b	38.25±0.45 a	38.35±0.18 a	38.45±0.02 a	38.38±0.05 a	38.32±0.03 a
PUFA	44.52±0.2 a	44.75±0.03 b	44.79±0.2 b	44.52±0.03 a	44.22±0.06 d	44.00±0.03 c
TFAs	0.05±0.00 b	0.21±0.01 a	0.28±0.01 a	0.43±0.01 c	0.62±0.04 d	0.84±0.01 e

Mean ± SD, n =3 expressed in relative percentage of total fatty acids. Data followed by different letters within each column are significantly different ($P < 0.05$). SFA, MUFA, PUFA and TFAs respectively denoted saturated, monounsaturated, polyunsaturated fatty acids and trans fatty acids.

Generally, FA composition after heat treatment depends on the type of seeds and process. For example, in hazelnut oil the level of SFA and of oleic acid were increased while that of linoleic acid was decreased (Amaral, Casal, Seabra, & Oliveira, 2006). In almond (*Prunus dulcis*) kernel, roasting increased the level of unsaturated FA (linoleic, oleic and elaidic acids) as well as saturated one (palmitic and stearic acids) with 150 or 180°C during 5, 10 or 20 min (Lin et al., 2016).

Sesame is a rich source of lipid containing high levels of UFA which have various health benefits (Hashempour, Ghazvini, Bakhshi, & Sanam, 2010). Compared to literature, after roasting, the reduction of PUFA rate (44.52 to 44%) was less than that reported previously with decreasing of PUFA from 46.5 to 25% in brown sesame (Hama, 2017). Indeed, the degradation rate of linoleic acid was relatively higher than that of oleic because the oxidation is more important as the level of double bound increases.

Trans fatty acids (TFAs) are produced by isomerisation of double bonds in FA, they increase cardiovascular disease and alter the HDL/LDL ratio. Roasting process had effected ($p<0.05$) TFAs, the total content varied from 0.20 to 0.84%. The maximum was recorded in RSSO at 300°C and the predominant TFAs produced was C18:2_C912T. Many studies have shown that TFAs can be formed in oil after roasting processes, it was observed that contents in TFAs such as C18:2t (n-6) and C18:3t increased gradually as roasting time increased at all temperatures used with a maximum content of 0.8% in perilla seeds (Zhao, Hong, Lee, Lee, & Kim, 2012).

Formation of TFAs depended on the type of roasted seeds: 0.95 g/100 g in pistachios, 0.50 g/100 g in peanuts, 0.90 g/100 g in almonds, and 0.60 g/100 g in sesame tahina (Yaacoub et al., 2008). The maximum of TFAs produced (0.84%) in our oil (20 min at 300°C) was higher than that reported by Amaral et al (2006) in hazelnut oil (0.07%) roasted at 145°C/15min, because sesame oil contained a high level of PUFS (44.52%) more sensible to oxidation after an intense heat treatment . However, it was equal to that find in perilla seeds roasted at 180 °C for 75 min (Zhao et al., 2012).

Production of TFAs including C18:2_T9C12 and C18:2_ C9T12 was linear (R^2=0.77; R^2=0.80, respectively), whereas production of C18:2_T9T12 was absent until 250°C, where it was linear between 210 and 300°C (R^2=0.99). A same linear increase (R^2=0.99) was reported as a function of time in sesame seeds (Yaacoub et al., 2008). Nevertheless, the presence of

TFAs in RSSO is not considered as harmful because their level is under the threshold allowed by the European regulation (max 2%).

I.2.7. Minor compounds

I.2.7.1. Lignans and tocopherols

Sesamin and sesamolin are the two major lignans detected in sesame oil. They exert many health-promoting benefits, such as anti-inflammatory, antioxidant, hypocholesterolemic, neuroprotective and antihpertensive activities (Rangkadilok et al., 2010); for this it is important to investigate their changes after roasting process. Sesamin was the major component in the unsaponifiable of sesame oil. Variation in sesamin and sesamolin contents is given in Table XIV. Statistical analysis revealed that sesamolin was more affected ($p<0.001$) by heat processing than sesamin ($p<0.01$).

The lignan level in oil before roasting was as follow: sesamin (393.2 mg/100g); sesamolin (202.92 mg/100g); these values were within the range already published (Rangkadilok et al., 2010;Yen, 1990; Yoshida & Takagi, 1997). Both of them were reduced to 344.19 and 147.12 mg/100g, respectively. Those values were obtained when seeds were roasted at 250 and 300°C, respectively. The reduction rate of sesamolin (27.50%) was greater than that of sesamin (12.47%), because sesamolin is decomposed into sesamol during roasting (Fukuda et al., 1986; Shahidi, 2004). sesamolin and sesamin content of sesame oil declined as the roasting temperature increased and no sesamolin was detected when seeds were roasted upper than 220°C (Yen, 1990).

Only γ-tocopherol was detected among tocopherol homologues in our samples. As it has been reported in the literature, 97% of the tocopherol in sesame oil was in the γ-form (Speek et al., 1985). USSO contains 25.93 mg/100 g; this quantity was in close agreement with the values previously reported : 28.93 mg/100 g (Yen, 1990); 38.62 mg/100g (Yoshida et al.,, 2001) and lower than that find by others: 40.4 mg/100g (Mohamed & Awatif, 1998); 57.6 mg/100g (Yoshida & Kajimoto, 1994).

After heat treatment, it was reduced to 23.18 mg/100 g. The higher loss (10.61%) was occurred when sesame seeds were roasted at 250°C (Table XIV). A reduction of less than 20% in γ-tocopherol was observed after sesame roasting for 25 min at temperature varied between 160 and 250°C (Yoshida & Takagi, 1997), Mohamed and Awatif (1998) found a reduction in

this compound from 40.4 to 33.0 mg/100g (Mohamed & Awatif, 1998). In hazelnuts, it was showed a loss in *γ*- and α-tocopherol below 10% and 9.2%, respectively at 185 °C during 15 min roasting (Amaral et al., 2006).

Table XIV. Sesamin, sesamolin and *γ*-tocopherol contentin raw and roasted sesame oils at different temperatures (expressed in mg/100 g of oil)

	Samples					
	USSO	**RSSO 150**	**RSSO 180**	**RSSO 210**	**RSSO 250**	**RSSO 300**
γ-tocopherol	25.93±1.5b	24.89±1.5ab	24.37±0.2ab	24.6.±1.5ab	23.18±1.6a	24.87±0.7ab
sesamolin	202.92±9.5a	201.351±14.3a	190.11±14.8ac	182.30±9.5c	163.37±9.9b	147.12±2.68b
sesamin	393.25±14.7b	392.4±4 b	364.89±10.8ab	361.56±9.3ab	344.19±13.73a	354.8±6.99 a

Results are mean ± SD, n =3. Values followed by different letters within each column are significantly different ($P < 0.05$).

A decrease in total tocopherol level was found during pumpkin roasting (Nađeral et al., 2012). In contrast, Vujasinovic et al. (2012) observed that pumpkin seeds roasting treatment increased more than 50% in the content of vitamin E (77.34 mg/kg), compared to the row seeds (50.21 mg/kg) (Vujasinovic, Djilas, Dimic, Basic, & Radocaj, 2012). In various cultivars from Iranian sesame, *γ*-tocopherol content increased with the roasting temperature and time; until 200°C/10 min, and then it was decreased by roasting at 220 °C for prolonged time (20 minutes) (Behrooz Jannat et al., 2013). They explained this effect by a heat breaking of bonds linking *γ*-tocopherol to proteins and phospholipids.

I.2.7.2. Sterol content and profile

The results for total and individual phytosterols are detailed in Table XV. Chromatographic quantification revealed that the main sterol constituents before and after heat processing were β-Sitosterol (48.42–50.55%), Campesterol (31.38–34.24%), Δ-5-Avenasterol (10.68–11.78%), Stigmasterol (5,06-5,2%) and small amounts (<2%) for other sterols. These values are in agreement with those previously published for *Sesamum indicum* (Itoh et al., 1973; Mohamed & Awatif, 1998).

β -sitosterol is the most abundant sterol, which represents approximately 50%. Among the various phytosterols, β -sitosterol has been the subject of much research concerning its beneficial and physiological effects on human health. The latter lowers cholesterol level

(Pegel, 1997), strengthens immunity and has anti-inflammatory, antipyretic and anti-carcinogenic effect (mainly prostate) (Klippell, 1997; Kritchevsky and Shirley, 2005).

Table XV. Sterol contents (relative % and total amount) of raw and roasted sesame seed oils at different temperatures

	Samples					
	USSO	**RSSO 150**	**RSSO 180**	**RSSO 210**	**RSSO 250**	**RSSO 300**
Campesterol	33.39±0.14c	34.06±0.14e	34.24±0.04f	33.8±0.04d	32.64±0.03b	31.38±0.05a
Stigmasterol	5.17±0.03ad	5.11±0.06cd	5.06±0.01b	5.1±0.03bc	5.2±0.02a	5.2±0.02a
β-Sitosterol	49.49±0.05b	48.44±0.15a	48.42±0.09a	48.65±0.05c	49.47±0.02b	50.55±0.01d
Δ-5-Avenasterol	10.68±0.03c	11.22±0.07ab	11.22±0.1a	11.33±0.06b	11.54±0.05d	11.78±0.1c
Δ-7- Stigmasterol	0.99±0.04b	0.95±0.07b	0.88±0.02ac	0.88±0.04a	0.93±0.03bc	0.88±0.00a
Δ-7- Avenasterol	0.22±0.03a	0.19±0.04a	0.14±0.02b	0.2±0.04a	0.2±0.03a	0.19±0.03a
Total sterols (mg/100g)	862.9±8.9b	896.4±10.9b	824.5±30.2a	819.1±9.6a	785.5±5.21d	740.2±8.54c

Results are mean ± SD, n =3. Values followed by the same letters within each column are not significantly different (P > 0.05).

Roasting temperature had affected ($p<0.05$) total and all the individual sterols. In fact, a slight decrease was observed in individual phytosterols of roasted samples varied between 2.69% and 11.11%. Whereas an important decrease was observed in both total phytosterols (17.42%) and Δ-7- Avenasterol (36.36%). Reduction in sterols level can result from their degradation by hydrolysis, oxidation or isomeration at high temperature (Piironen et al., 2000). Δ-5-Avenasterol was the only phytosterol that increased linearly (9.33%) compared to the value of untreated sesame ($y=-0.003x+10.65$; $R^2=0.97$). It has been showed that Δ-5- and Δ-7-avenasterols were increased whereas β-Sitosterol and campesterol were decreased in white and brown sesame seeds roasted at 180°C for 30 min (Mohamed & Awatif, 1998).

Total sterol content of sesame was found to be high (>800m g/100g) compared to some other vegetable oils like olive, sunflower, soya and peanut (Kamm et al., 2001)**.** We know that, some sterols including Δ-5-Avenasterol had an antipolymerization effect which participates during heat treatment to the protection of oil compounds from oxidation (Akintayo, 2004). In our samples Δ-5-Avenasterol was increased in heated samples; this can be another argument for the stability of sesame oil at high temperatures

I.2.7.3. Carotenoids

Carotenoids contribute to the color of oils and fats but they also protect them against oxidation. The amount of total carotenoid pigments of raw and roasted *Sesamum indicum* (Figure 24) was generally low, and ranged from 0.09 to 0.28 mg/100 g of oil. The optimum yield of carotenoids was obtained at 150°C. At 300 °C, we have produced a loss of more than 65 % of carotenoid.

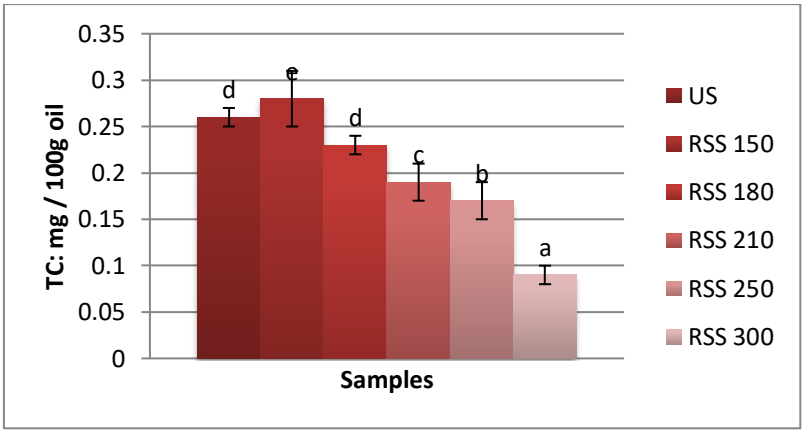


Figure 24: Total carotenoids of sesame seeds oil. Values followed by different letters are significantly different ($P < 0.05$).

I.2.7.4. Total phenolic content

As it is shown in figure 25, phenolic content was increased with increasing in roasting temperature, describing a linear curve: $y=1.39x+1474$; $R^2 =0.90$. The persistence of the increase in TPC at high temperature indicates that sesame phenolics are heat-resistant. In our experimental conditions, treated seeds with high temperature showed a significant increase of 21.49% in TPC compared to USSO. Several authors reported improvements in TPC by heat treatment mainly after roasting; an increase of TPC , from 70.953 µM to 129.3 µM after roasting at 200 °C for 20 min was observed in Naz-Branching sesame (Jannat et al., 2013). Another study demonstrated that, roasting increased TPC in both the soluble and bound extracts in whole nut kernel (Chandrasekara & Shahidi, 2011b).

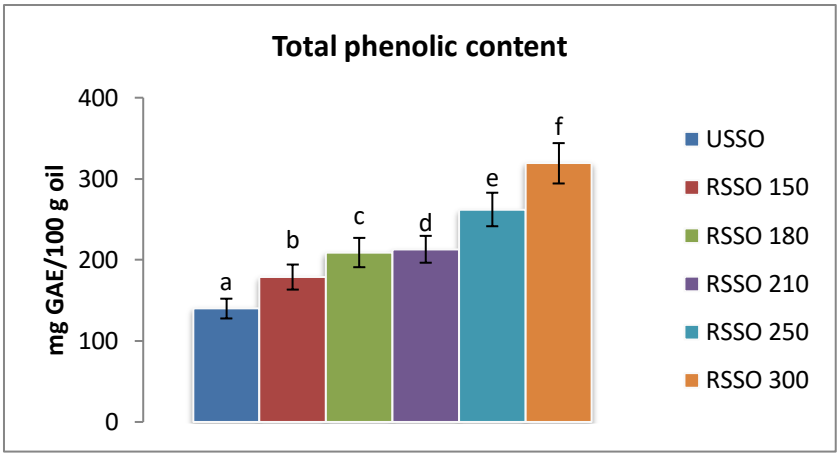


Figure 25: Total phenolic content of sesame seeds oil. Values followed by different letters are significantly different ($P < 0.05$).

I.2.8. Antioxidant activity by DPPH test

Results of antioxidant activity evaluated by the DPPH test of different sesame oils are shown in figure 26. Comparatively to USSO, all the RSSO tested showed slightly higher ($P < 0.001$) antioxidant activity, which confirms the results find with Rancimat test. Indeed, the antioxidant capacity of RSSO (319.09 mg GAE/ 100 g oil) at 300°C was 3 times higher than that of USSO (139.86 mg GAE/ 100 g oil).

Several studies were undertaken to evaluate the effects of seeds roasting on the antioxidant activity and TPC. Jannat findings for sesame oil are in agreement with our results, in which ferric reducing antioxidant power assay and TPC increased significantly as the roasting temperature was higher (Jannat et al., 2010). Indeed, nuts cashew, peanut and hazelnut roasting treatment improved TPC as well as antioxidant activities of the tested samples (Vinson & Cai, 2012; Chandrasekara & Shahidi, 2011b).

Namely, a very significant correlation was found between the TPC and DPPH (R^2=0.9773), as well as between the IP of Rancimat and TPC (R^2=0.84), indicating that these compounds participate strongly to the prevention of oil oxidation.

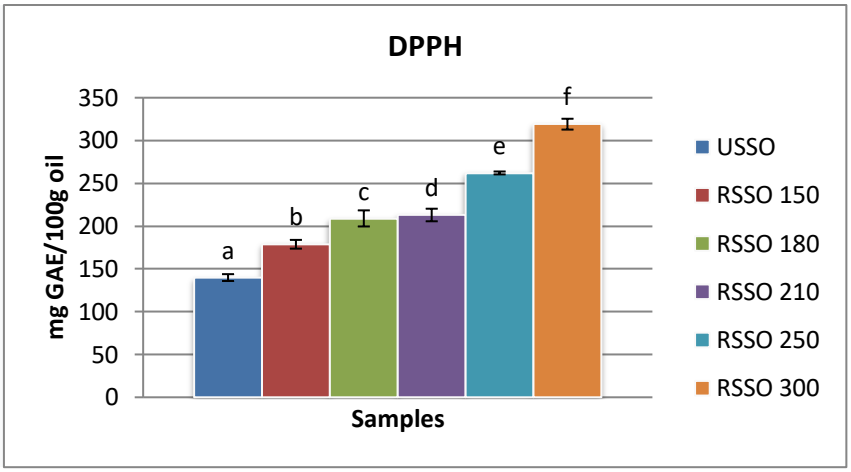


Figure 26: Antioxidant activity of sesame seeds oils. Values followed by different letters are significantly different ($P < 0.05$).

Conclusion (part I)

This first part of our experimental work aimed to determine the effect of roasting temperature on (i) seed composition , (ii) sesame seeds extracted oil characteristics including changes in composition (fatty acid profile ,lignan, sterol and tocopherol content), organoleptic and physico-chemical properties as well as its quality (polar compounds formation). Oxidative stability and antioxidant capacity was also evaluated in raw and roasted sesame seeds oils.

Our results showed that sesame seeds are rich in nutrients: they contain a high quantity of oil (48.43%), as well as 22.38 % protein, 15.41% carbohydrate and 5.67% ash. After seed roasting, a significant reduction in the level of proteins (3.6%), moisture (25.6%), cellulose (4.0%) and ash (24.5%) were observed. But in the case of fat (lipids) and ash it was observed a significant enhancement at 180°C and 250°C.

The oil content is higher than most of the known oilseeds. Analysis of fatty acids revealed that it belongs to linoleic-oleic (44.12/37.55%) acid and contains 16.24% saturated, 38.94% mono-unsaturated, and 44.52% poly-unsaturated fatty acids. The oil was rich in lignans and sterols as it contains 393 mg/100g of sesamin, 203 mg/100g of sesamolin and 863 mg/100g of total sterols. The most abundant sterol was β-Sitosterol (49.5 %) and Campesterol (33.4%).

There was no obvious difference in some physico-chemical proprieties of extracted oils including density and refractive index, but the saponification values, acid value and iodine value were affected by temperature. However refractive index and iodine value decreased suddenly when the roasting temperature was above 250°C, resulting from reducing in saturation level. An important increase in red color and decrease in light color was observed at high temperature (> 210°C°). Roasting had also gived a nutty flavor in seeds and their corresponding oils and develop color by Maillard reaction products

There were an important primary and secondary lipid oxidation at T> 250°C, resulting in higher *p*-anisidine value and K232 as well as K268 values. Roasting improved oil yield, increased resistance to oxidation by enhancing induction period (from 5.51 to 10.50 h), enhanced total phenolic content (from 152.2 to 193.9 mg/100 g) and antiradical activity of the extracted oil.

A gradual decline was observed in total phytosterols (maximum of 17.4%), *γ*-tocopherol (maximum of 10.6%), sesamolin (maximum of 27.5%), sesamin (maximum of 12.5%) and total carotenoids (maximum 65 %). All the extracted oils presented weak triglyceride polymers content, in agreement with the European regulation.

Regarding the studied parameters, the optimum roasting temperature is suggested to be at 210°C for 20 minutes, to have the better sesame oil with a minimum oxidation and a healthy quality.

It was also observed the liberation of free fatty acids at high temperature (300°C) by hydrolysis of mono-, di-, and triacylglycerols; the increase in free fatty acids content can accelerate the process of oil oxidation and leads to poor oil quality.

II. Impact of sesame seed addition on quality parameters, probiotic viability and sensory properties of stirred yogurt

II. 2. Materials and methods

II.2.1. Sample preparation

Sesame seeds (*Sesamum indicum*) used in this section are the same studied previously in the first experimental part. One part of the seeds was roasted at 180°C for 20 minutes using an electric oven. Unroasted and roasted seeds were ground (GM 200; Retsch GmbH, Germany) to achieve particle size of 2-3 mm then stored in sealed plastic bags (4 °C) until use, to avoid oxidation.

The freeze-dried mixture of starter culture (*Lactobacillus delbrueckii* ssp.*bulgaricus* and *Streptococcus salivarus* ssp.*thermophilus*) and the probiotic strain (*Bifidobacterium animalis*ssp. *lactis*, BB-12) were purchased from CHR Hansen (France).

II.2.2. Determination of the optimum of sesame incorporation

Pasteurized milk was mixed with skim milk powder to have milk with 18 % total solids and stirred at 85 °C for 20 min. After cooling to 40 °C, the starter culture was incorporated and the milk was divided into conical tubes (50 mL). Variable concentrations of ground sesame seeds (0-10%, w/v) were added in order to determine the best incorporation rate that can be added to milk before fermentation without producing syneresis and disrupting bacterial growth (Agil *et al.*, 2013). Incubation was carried out at 40–42 °C until pH 4.5.

II. 2.3. Yogurt manufacture

Yogurt was prepared in a laboratory scale using the method described previously (Kailasapathy *et al.*, 2008). Pasteurized milk (3.5% fat) was purchased locally from a commercial source (Candia, Bejaia). Skim milk powder (SMP) was added with speed stirring, to have milk with 18 g/100 g total solids. Then it was heated to 85 °C for 20 min and cooled quickly to 40 °C. Commercial sugar (8%) and 2 g/100 g (w/w) of frozen yogurt starter culture was incorporated and the mixture stirred continuously then divided in 150mL plastic yogurt cups.

Freeze-dried culture of *B. animalis ssp. lactis* (10^8cfu/g) 2 g/100 g (w/w) was incorporated and sesame seeds (6 %) added to make six yogurts: Y, YP, YUS, YRS, YPUS, and YPRS (Table XVI).

Incubation was carried out at 40–42 °C until a pH of 4.5. After fermentation yogurts were quickly cooled in ice water bath then stirred. Samples were prepared in triplicate and the yogurt cups were sealed then stored in the refrigerator at 4 °C for 28 days for analysis.

Table XVI: Recipe of standard or control yogurt (Y), probiotic yogurt (YP), unroasted sesame yogurt (YUS), roasted sesame yogurt (YRS), probiotic unroasted sesame yogurt (YPUS) and probiotic roasted sesame yogurt (YPRS).

Samples	Total solid %	Lactic ferment %	Sugar %	Probiotic %	Sesame seed %
Y	18	2	8	0	0
YP	18	2	8	2	0
YUS	18	2	8	0	6
YRS	18	2	8	0	6
YPUS	18	2	8	2	6
YPRS	18	2	8	2	6

II. 2.4. Enumeration of probiotic and starter cultures

Bacterial count was carried out weekly for a total of four weeks (days 1, 7, 14, 21, and 28). For each lot at different dilutions (four to five serial dilution of 1/10), 100µL of each of the last three dilutions were spread by the streaks method on Petri dishes. For enumeration of starter cultures, the inoculated media were incubated at 40 °C for 24 h (Espírito Santo *et al.*, 2010). The probiotic was enumerated on MRS agar containing L-cysteine hydrochloride (0.5 g/L) and incubated (40°C, 72h) under anaerobic conditions (Rodrigues *et al*., 2012).

Plates containing 30-300 colonies were enumerated and the results were expressed as log colony forming units per milliliter (log cfu/mL) using the following equation:

cfu= number of colonies/plate factor x dilution factor (Sun-Waterhouse *et al.*, 2013)

Where plate and dilution factors refer to the amount of sample pipetted and the dilution series of the yogurt sample, respectively.

II.2.5. Determination of pH and total titratable acidity (TTA)

pH was measured at 25°C by electrode immersion with a pH meter (211 HANNA) each week until the 28th day. Yogurt (1 mL) was mixed with distilled water (9 mL) with a few drops (3 to 5) of phenolphthalein 0.1% (w/v). The titration was made with a solution of NaOH (0.1N) until the persistence of a pink color. The volume of NaOH used for titration was noted and the titratable acidity (TTA %) was expressed as a percentage lactic acid equivalent and calculated according to the following equation:

TTA (%) = V_{NaOH} × 0.1N × 100% × 0.009 × 10 (dilution factor)

Where V_{NaOH}: Volume of NaOH in mL used for titration and 0.0090 is the coefficient corresponding to lactic acid.

II.2.6. Proteolytic activity

Proteolytic activities of organisms used in yoghurts were assessed by measuring liberated amino acids and peptides using the o-phthaldialdehyde (OPA) method (Donkor *et al.*, 2007)**.** 2.5 mL of yoghurt samples were added to 5 mL of 0.75% (w/v) trichloroacetic acid and the mixture was vacuum-filtered with filter paper. 150 mL of the permeate was mixed with 3 mL of OPA reagent and the absorbance of the solution was measured using a spectrophotometer after 2 min at room temperature at 340 nm (20° C).

The proteolytic activity of yogurt bacteria was expressed as the absorbance of OPA derivatives. The concentration of free amino groups and peptides is proportional to the absorbance at 340 nm.

II.2.7. Syneresis

Syneresis was determined using the centrifugation method (Aprianita *et al*., 2009). 10 g of yogurt from different days storage (1, 7, 14, 21, 28) was centrifuged (Bench-top centrifuge NF 200, Belgium) 700×g at 8 °C for 10 min. The clear supernatant was weighed, and syneresis was expressed as percent weight of supernatant relative to the original yogurt weight using the following equation:

Syneresis (%) = (weight of whey collected/weight of yogurt)X 100

II.2.8. Total phenolic content

First water yogurt extracts were prepared using the method of (Amirdivani & Baba, 2011). 10 g of yogurt was mixed with 2.5 mL of distilled water; the mixture was stirred, and adjusted to pH4 with HCl solution (0.1 M). The mixture was heated in a water bath (45 °C, 10 min) and centrifuged (5000 g, 10 min, 4°C) to remove precipitated proteins. The supernatant was adjusted to pH7 with 0.1M NaOH and centrifuged (5000 g, 10 min) to remove residual proteins and salts then stored at -20 °C for later use. Extractions were carried out in triplicates.

The total phenolic content was determined using the method of (Shetty *et al.*, 1995). 1 mL of water yogurt extract from different storage days (1, 7, 14, 21, and 28) was mixed with 1 mL of 95% ethanol and 5 mL of distilled water. Folin–Ciocalteu reagent (0.5 mL) was added followed by thorough mixing. After 5 min, 5% Na_2CO_3 (1 mL) was added and the reaction mixture was allowed to stand for 60 min at room temperature.

The absorbance was monitored at 725 nm and total phenolic compounds expressed in microgram equivalent of gallic acid per gram (µg GAE/g) sample using a standard curve established under the same condition using various gallic acid concentrations in methanol.

II.2.9. Antioxidant activity by DPPH inhibition assay

The antioxidant capacity of yogurt extracts was determined with the method described by (Behrad *et al.*, 2009).Yogurt water extract (250 µL) was mixed with ethanol solution of 60 µM DPPH (3 mL). The mixture was shaken vigorously and then incubated for 20 min at room temperature in the dark. The absorbance was measured at 517 nm. The readings were compared with the control which contained distilled water instead of yogurt extract. The % inhibition of DPPH was determined using the following equation:

$$\% \text{ inhibition} = (A_{control} - A_{sample})/A_{control} \times 100.$$

II.2.10. Yogurts Sensory evaluation

Ten untrained panelists familiar with yogurt which are students in food sciences and technology from the University of Bejaia, Algeria; were selected for sensory evaluation. The sensory analysis was based on a nine-point 1 (poor) to 9 (excellent) hedonic scales for some sensory parameters including color, odor (flavor), taste, texture, acidity, syneresis and overall acceptability (Haque & Ji, 2003).

The stored yogurt samples were evaluated by nonsmoker's panel members on 1st day of storing. Water and bread were provided for the panelists between samples to cleanse the palates.

II.2.11. Statistical analysis

Three determinations were made for all the assays. Analysis of variance was performed by the general linear models (GLM) procedure, means comparison by Duncan's test, and Pearson correlation according to Statistical Analysis System, SAS 9.1 for Windows.

II. 3. Results

II.3.1. Optimum of sesame incorporation

Yogurts prepared with an incorporation rate of 8% and 10% have a liquid consistency and texture like a yogurt drink which is due to the phenomenon of syneresis, this will be not accepted by consumers, but yogurts containing 0 to 6% of ground sesame have a consistent texture. Consequently, the best concentration was 6% of ground sesame which can be incorporated into yogurt before fermentation under our experimental conditions without syneresis or whey separation from gel.

II.3.2. Bacterial counts

Figure 27 shows the variation in bacterial counts of starter culture in the six preparations of yogurt. Bacterial count was affected by probiotic (P<0.0001), storage time (P<0.01) and their interaction (P<0.001). Sesame and probiotic exerted beneficial effects on the control yogurt (Y) culture (P< 0.05). On the 1st day of cold storage, *S.thermophilus* and *L. bulgaricus* counts did not differ and varied from 8.12 to 8.39 cfu/mL; then it declined to 7.45-8.35 log cfu/mL. Reduction rates varied from 0.47 to 8.28 % in the following order: Y>YUS>YRS>YPRS>YPUS>YP. In fact, bacterial counts decreased linearly in Y (Y =-0.18x+8.27; r^2=0.93) and YUS (YUS =-0.14x+0.37; r^2=0.98).

From day 14 to day 28, the highest count of starter culture was recorded in probiotic yogurt (YP) and the viability was considerably stable and maintained during this storage time. At day 7, the number of counts increased by 2.8 % in YPUS followed by a sharp decline at the 14 day. The presence of probiotic bacteria supported and helped growth of yogurt starter

culture, even in the absence of sesame; this can be explained by a competition of starter culture with the probiotic for the nutritive elements by increase in the number of living cells. Significant enhancement of *L. delbrueckiis sp. Bulgaricus* counts was reported in yogurt made with a mixture of probiotic strains (*Lactobacillus acidophilus, Bifidobacterium lactis and L. para casei*) (Donkor et al., 2006). An important increase (15-20 %) was observed in *S. thermophilus* counts during probiotic yogurt storage, but decreased after 21 day from 7 to 33% (Dave and Shah, 1997). *L. bulgaricus* counts also increased initially then declined during the later stage.

The population counts were similar for YPUS-YPRS and YUS-YRS, without significant differences particularly at the beginning and end of storage indicating similar behavior of yogurt bacteria in the presence of raw or roasted ground sesame.

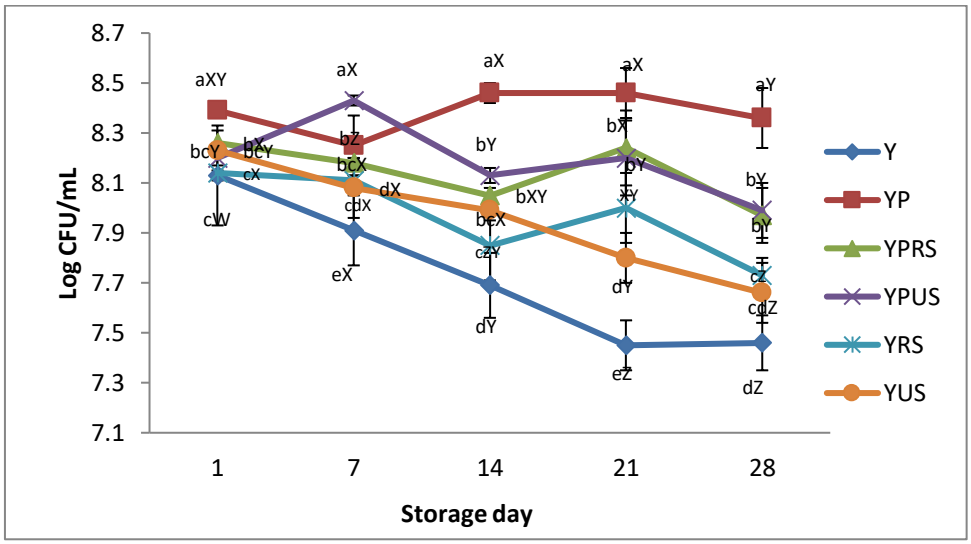


Figure 27: Microbial viability of starter culture in all the yogurt preparations during 28 days storage at 4°C. Means with different lower and upper case letters are significantly different ($P< 0.05$).

The viable counts of *B. lactis* (log cfu/mL) during the four weeks of cold storage (Figure 28) was significantly ($P<0.001$) affected by sesame supplementation and storage time. Except on day one, where the difference was insignificant, probiotic supplemented yogurts with roasted or unroasted sesame exhibited higher *B. lactis* ($P<0.05$) counts compared to the control (YP) demonstrating its prebiotic potential.

Probiotic count was within the 7.83 to 8.36 log cfu/mL range in unsupplemented yogurt (YP) with optimum at day 1 and 7.73 to 8.44 log cfu/mL in supplemented yogurt with an optimum at day 14 and 21 for YPUS and YPRS, respectively.

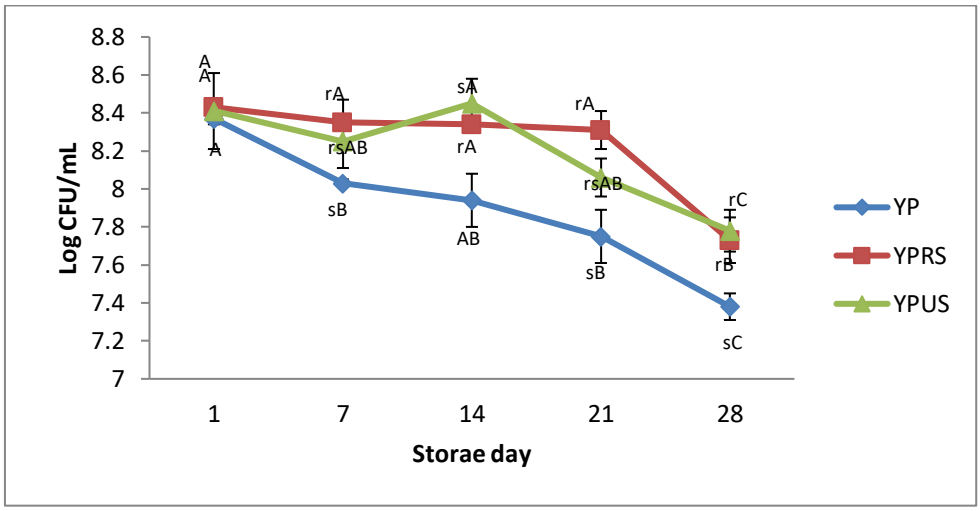


Figure 28: Microbial viability of *B. lactis* in the probiotic yogurts during 28 days storage at 4°C. Means with different lower and upper case letters are significantly different ($P< 0.05$).

The number of probiotic colonies varied between the supplemented yogurts (YPUS and YPRS); but difference on day 1 and 28 was not significant between them. Probiotic growth decreased initially for the first week for both supplemented yogurts, then increased in YPUS and remained stable until 21 day in YPRS. From day 1 to 28, in unsupplemented probiotic yogurt (YP) the number of viable count decreased linearly by 1 log cfu/Ml (YP=-0.22x+8.56; r^2=0.95). At 28 days, all sesame yogurts had significantly higher probiotic (P<0.05) counts compared to the control by over 0.34 log cfu/mL.

Ground sesame (*Sesamum indicum*) addition promoted total bacterial organisms of *B.animalis ssp Lactis* probably due to their improved survival by providing essential nutrients and oligosaccharides. Probiotic strains are recognized to have nutritional requirement and important proteolytic activity to support their growth. For this, they need free amino acids to increase their viable cells (Vasiljevic & Shah, 2008). Indeed, proteins are the second predominant compounds in sesame seeds (24 g/100 g) that is rich in methionine, cystine, arginine, and leucine (36, 25, 140, and 75 mg/g protein, respectively) (Namiki, 2007). Sesame is an excellent source of essential amino acid significantly higher than that of FAO/WHO requirement for human except lysine (Johnson et al., 1979).

The high (18-20%) carbohydrate content of *Sesamum indicum* (11% dietary fiber) with low glucose, fructose, low starch amounts and the presence of an oligo sugar planteose (O-α-D-galactopyranosyl-(l,6)-*β*-D-fructofuranosyl-α-D-glucopyranoside) unaffected by the human digestive enzymes maybe responsible for the prebiotic potential of sesame (Namiki, 1995). During the storage time, the viability of *B. animalis* in probiotic yogurts was superior to the minimum recommended level (>6 log CFU/g) to provide the beneficial health effects to the gut (Kongo, Gomes, & Malcata, 2006).

The viability loss/reduction in probiotic bacteria may be due to post-acidification during refrigerated storage (Sun & Griffiths, 2000). The co-culture of starter culture with bifidobacteria which are proteolytic species is not recommended because they create acidic conditions (Abu-Taraboush, Al-Dagal, & Al-Royli, 1998). Some probiotic strains are sensitive to antimicrobial substances produced by yogurt bacteria during refrigerating storage (Nayan Shah, 2001). Our results showed that raw and roasted sesame may selectively impact probiotic growth with very low effect on yogurt starter culture especially at the long cold storage period (14-28 day).

3.3. Post-acidification (pH) and titratable acidity

Yogurt pH were significantly affected by sesame supplementation ($P< 0.0001$) and storage time ($P< 0.01$). Yogurt bacteria metabolize carbohydrates for growth and energy producing various organic acids such as lactic, butyric, propionic, acetic and citric acids (Fernandez-Garcia & McGregor, 1994). pH is a measurement of hydrogen (H^+) concentration contributed by the released organic acids during fermentation and storage.

At day 1, the pH among all yogurt treatments ranged from 4.41 to 4.52 (figure 29), then it decreased linearly until 3.68 to 3.91.There was no significant difference ($P >0.05$) between unsupplemented yogurts (Y and YP) during storage except on day 14. From day 1 to day 14 there were no differences between all the supplemented yogurts with or without probiotic, indicating that probiotic had no effect on pH ($P> 0.05$).

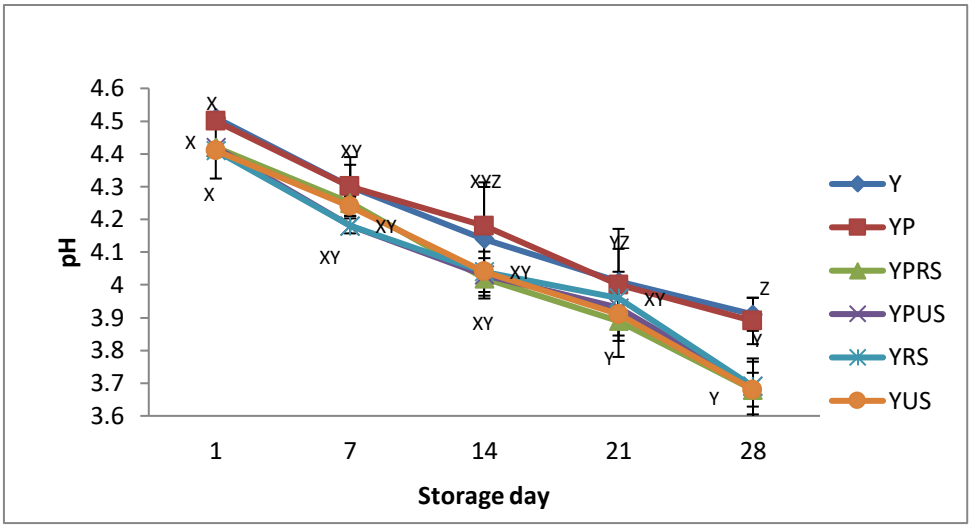


Figure 29: Post-acidification in yogurts during 28 days storage at 4°C. Means with different lower and upper case letter are significantly different ($P < 0.05$).

A similar gradual reduction in pH (from 6.55 to 4.31) was reported by Leite et al, (2013). This would be a consequence of the production of organic acids which contribute to the flavor, aroma and preservation of fermented dairy products. This reduction in pH would be favored by the components of sesame seeds (containing easily fermentable polysaccharides) without affecting the fermentation capacity of the microflora.

TTA measurement is an indicator of bacterial metabolic activity in fermented dairy product. At day 1 the TTA values ranged between 1.6 and 1.85 then increased until 3.21 and 5. TTA for the four sesame yogurts was higher than that of control and probiotic yogurts (Y and YP) during storage, the evolution of titratable acidity in those yogurts (YUS, YRS, YPUS, YPRS) may be described in two distinct phases (figure 30). Between day 1 and 14, there was a gradual significant increase and between day 14 and 28 the increase was greater and rapid (YUS=0.27x+1.15; r^2=0.99; YPRS=0.83x+1.02; r^2=0.97).

The addition of both raw and roasted ground sesame was helpful in increasing total titratable acidity ($P< 0.01$) during storing indicating that microorganisms was more active in the presence of sesame seeds. Sesame contains various carbohydrates including D-glucose (3.63%), D-fructose (3.43%), D-galactose (0.40%), sucrose (0.17%), raffinose (0.59%), stachyose (0.38%), planteose (0.23%), and sesamose (0.14%) which could be easily metabolized by yogurt bacteria (Wankhede & Tharanathan, 1976).

The reduction of pH and liberation of lactic acids reflects the high metabolic activity of yogurt bacteria. Lactic acid bacteria (LAB) use milk proteins (nitrogen source) and carbohydrate from sesame seeds (carbon source) to produce organic acids and other volatile compounds including acetaldehyde and diacetyl. Organic acids play an important role as natural preservatives and the volatile compounds contribute to the organoleptic properties and yogurt flavor (Fernandez-Garcia & McGregor, 1994).

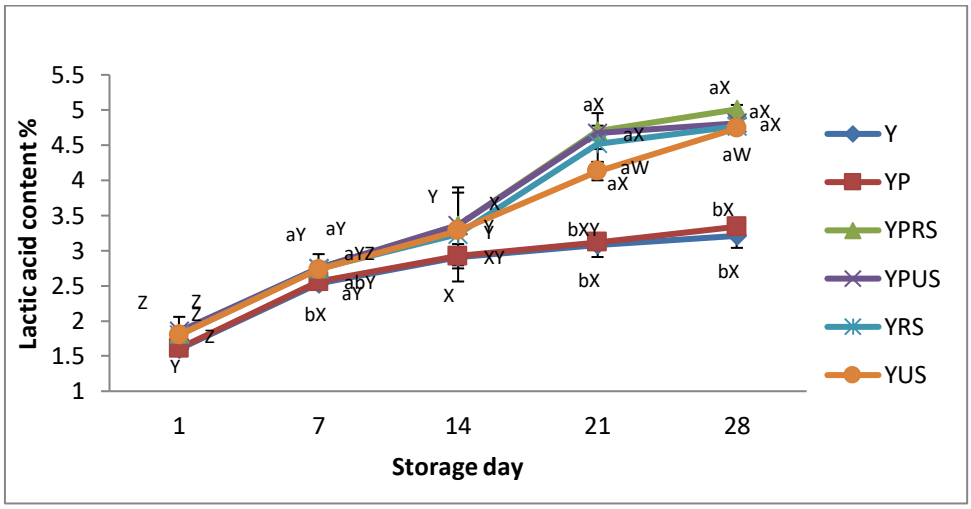


Figure 30 : Titratable acidity of all yogurt samples over the cold storage. Means with different lower and upper case letter are significantly different (P < 0.05).

The characteristics of yogurt (high microbial growth and acidity and reduced pH) improved by ground Sesame supplementation during storage may enhance the bifidogenic / probiotic effect such as resistance to infection of gastrointestinal pathogens, stimulate protein digestion and advantageously contribute to taste unique, aroma and flavor.

II.3.4. Proteolysis by o-phthalaldehyde (OPA) assay

Proteolytic activity improved significantly (P<0.05) in the presence of probiotic organisms and sesame seeds (Table XVII). All yogurts had higher OPA values (0.9-1.04) than that of the control yogurt (0.62) at the beginning and during refrigerated storage. The highest proteolytic activity (1.26) was recorded in both supplemented yogurts (YRS and YUS) at day 14 and day 21, respectively.

Proteolytic activity of bacteria was similar in probiotic sesame containing yogurt (YPUS and YPRS) indicating that seed roasting had no effect on yogurt proteolysis (P>0.05).

Proteolysis increased in all yogurts until the optimum absorbance recorded on day 14 or 21 depending on yogurt sample then it decreased at the end of storage. Enhanced proteolytic activity suggest that enzymatic activity of protease from starter culture and probiotic was markedly improved in the presence of ground sesame seeds that provided divalent ions including $Ca^{2+,}$ Fe^{2+} and Mg^{2+}. Those minerals were reported to be necessary for protease activity (Llorente-Bousquets et al., 2008). Indeed, sesame seed was found to be rich in various minerals. Among them, calcium and iron, which are often deficient in modern diets, and they are present in high concentrations (1200 and 9.6 mg/100 g, respectively) (Namiki, 2007).

Table XVII: Proteolysis (OPA) of yogurts during 28 days storage at 4°C

	Storage time (Days)				
Sample	**1**	**7**	**14**	**21**	**28**
Y	0.62 ± 0.01^{cZ}	0.78 ± 0.02^{cXY}	0.85 ± 0.02^{dXY}	0.87 ± 0.01^{dX}	0.74 ± 0.03^{cY}
YP	0.90 ± 0.01^{bY}	0.99 ± 0.06^{bXY}	1.12 ± 0.02^{cX}	1.04 ± 0.03^{cXY}	0.99 ± 0.04^{bXY}
YPRS	1.01 ± 0.02^{abZ}	$1.13ab\pm0.04^{XYZ}$	1.25 ± 0.06^{abX}	1.07 ± 0.08^{bcYZ}	1.14 ± 0.02^{aXY}
YPUS	1.04 ± 0.04^{aZ}	1.17 ± 0.06^{aXY}	1.22 ± 0.01^{abX}	$1.08b\pm0.03^{cYZ}$	1.08 ± 0.08^{abYZ}
YRS	0.93 ± 0.01^{abY}	1.19 ± 0.02^{aX}	1.27 ± 0.05^{aX}	1.17 ± 0.03^{abX}	1.16 ± 0.07^{aX}
YUS	0.98 ± 0.03^{abZ}	1.06 ± 0.02^{abYZ}	1.18 ± 0.06^{bcXY}	1.26 ± 0.02^{aX}	1.14 ± 0.01^{aXY}

Results are expressed as absorbance at 340 nm; means with different lower and upper case letters in the same column or row are significantly different (P < 0.05).

II.3.5. Syneresis

Syneresis is an undesirable feature in yogurt resulting from the separation of liquid phase (serum) from the protein gel (Shah, 2003). Syneresis of investigated yogurts was significantly affected by sesame supplementation (P<0.001) and storage time (P<0.001) but there was no difference between roasted and unroasted sesame supplementation (P>0.05).

At day 1, syneresis varied between 41.63 and 42.26 % (figure 31) in all yogurts without significant difference (P>0.05). Whey separation of yogurts containing sesame except YPUS followed parallel biphasic trends during storage with inflection at 14 days.

From day 1 to 14, the difference in syneresis level was insignificant among the three supplemented yogurts (YUS, YRS, YPRS), compared to control (Y) and probiotic yogurt (YP). Storage linearly increased whey separation (14-28 day) especially in supplemented yogurts (YUS=2.63x+41.8; r^2=0.96;YRS=3.33x+40.6; r^2=0.98, YPRS =2.5x+41.33; r^2=0.99).

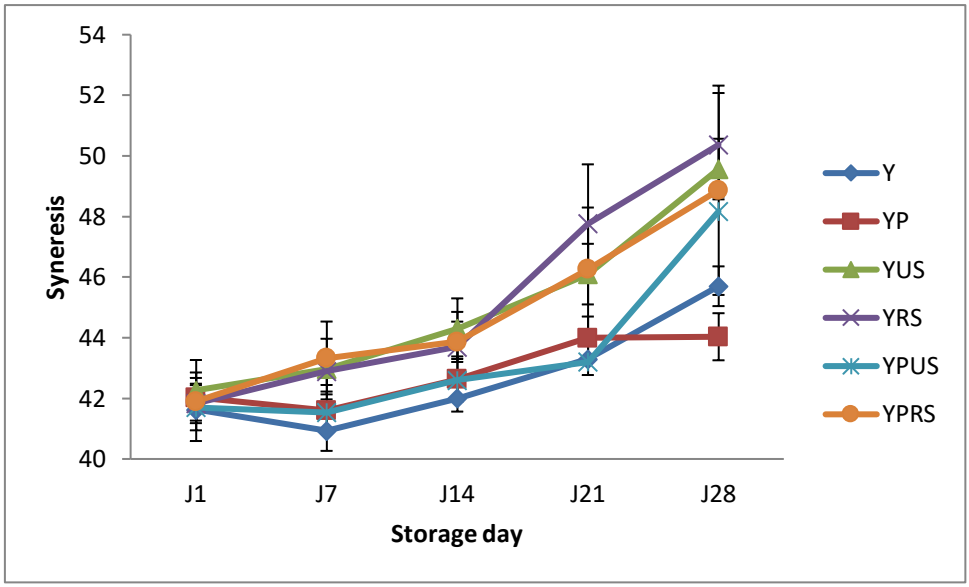


Figure 31: Syneresis of yogurts during 28 days storage at 4°C. Results are expressed in% of collected whey; means with different lower and upper case letters are significantly different (P< 0.05).

Syneresis increased with sesame addition compared to control and probiotic yogurts (figure 31). Many studies have reported higher syneresis accompanied with low viscosity for enriched yogurts with various plant ingredients including fruits or seeds. Therefore, addition of fruits decreased water-holding capacity of protein and viscosity resulting in increases syneresis (Akyüz & Coflkun, 1995; Mohamed et al., 2014;Zainoldin & Baba, 2009). At the end of cold storage, control and probiotic yogurts (Y and YP) manifested the lowest syneresis percentage (45.7and 44.03 %, respectively) whereas the highest mean value (50.36 %) was recorded in roasted sesame containing yogurt (YRS).

The syneresis trend of these yogurt paralleled changes in their proteolysis (OPA) and TTA, thereby indicating their strong association. Therefore, increased syneresis in sesame containing yogurts can be attributed to the milk protein degradation by lactic acid bacteria which are responsible for water retention.

II. 3.6. Total phenolic content (TPC)

Phenolic content did not differ significantly between control (Y) and probiotic yogurt (YP) and between the sesame containing yogurts (figure 32). The presence of PC in unsupplemented yogurts (Y and YP) can result from the phenolics linked to cow milk proteins (Besle et al., 2010) or to the presence of other reducing substances which respond to the photometric total phenolic estimation; indeed, tyrosine (with a phenolic side chain) was reported to elevate the reading in TPC measurement (Shah, 2000).

In the first week of storage, TPC increase for both YUS YPUS, it was reported that lactic acid bacteria of yogurt use phenolic acids such as ferulic and *p*-coumaric acid during fermentation process and post acidification and lead to the production of other phenolic acids such as vanillic and *p*-hydroxybenzoic acids before the aromatic ring structure is broken down (Blum, 1998).

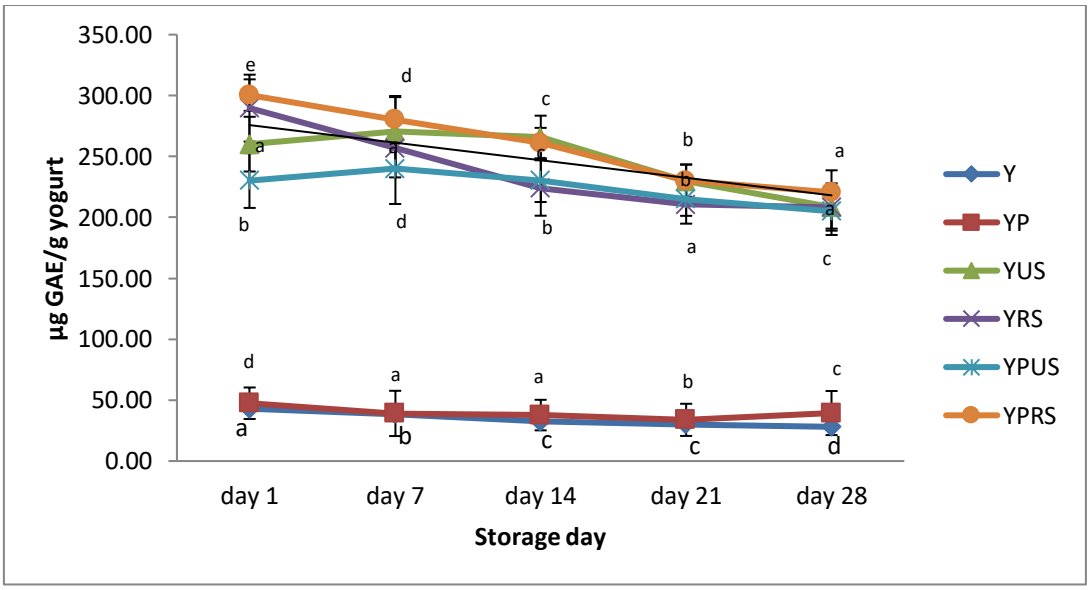


Figure 32: Total phenolic compounds of control and probiotic enriched yogurts during cold storage. Means with different lower and upper case letters are significantly different ($P< 0.05$).

Sesame increased (5-6x) phenolics content of control yogurt with or without probiotic. Similar increase (10x) in phenolic content has been reported for mulberry fruit fortified yogurt compared to control (Sigdel et al., 2018). Fortified yogurts with grape seed and garlic water extracts contained more total PC in all the samples compared to their controls (Chouchouli et al., 2013; Shori & Baba, 2014).

Roasting treatment had enhanced PC for 11 and 30% in both enriched yogurts with sesame (YRS and YPRS respectively). Storage significantly ($p< 0.001$) reduced phenolic contents of control and probiotic fortified yogurts linearly (Y = -0.55x+42.38; r^2 = 0.95 and YPRS = -3.08x+302,1; r^2 = 0.98). At 28 day storage PC reduction in the six yogurts varied between 16 to 34%. According to Oliveira et al., (2015) in strawberry fortified yogurt, reduction in PC were due to their association with serum milk proteins such as β-lactoglobulin (β-LG) and α-lactalbumin.

II.3.7. Antioxidant activity

Antioxidant capacity of the six yogurt preparations during storing at 4°C is summarized in figure 33. Yogurts containing raw or roasted sesame displayed strong scavenging capacity, whereas control and probiotic yogurts (Y and YP) manifested lower scavenging capacity. Our results agreed with the finding of Hashish et al (2014) where the antioxidant activity of yogurt increased with white and red sesame tahina (1 to 6%) supplementation (Hashish, Mohammd, & Salem, 2014).

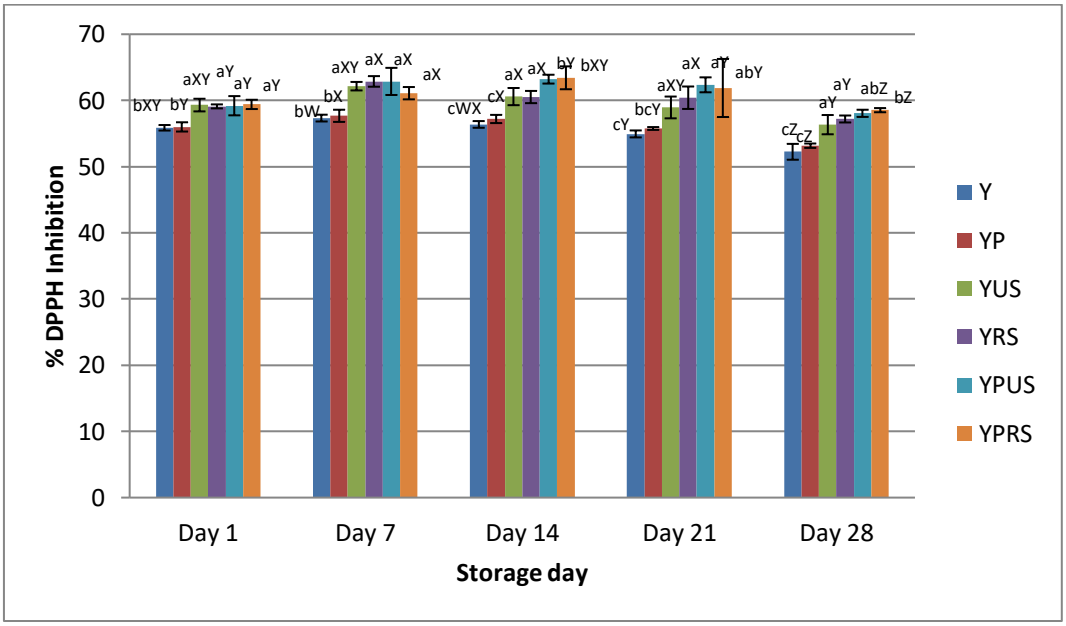


Figure 33 :DPPH radical scavenging activity of yogurts during 28 days storage at 4°C

Means with different lower and upper case letters are significantly different ($P< 0.05$).

Many studies reported higher antioxidant capacity of yogurt supplemented with various ingredients from plant material (fruits, leaves and seeds) compared to their control (Muniandy

et al., 2016; Sah et al., 2016; Van Nieuwenhove et al., 2019). This potential was attributed to the content of phenolic compounds (Baba, Najarian, Shori, Lit, & Keng, 2014). In our study, proteolysis was more marked in yogurts enriched with sesame seeds. In addition, bioactive peptides released by lactic acid bacteria during proteolysis may play an important role as antioxidant compounds.

II.3.8. Yogurts Sensory evaluation

The results of the sensory evaluation after 1 day of refrigerated storage performed in all yogurt samples are reported in figure 34. Roasted sesame containing yogurt (YRS and YPRS) was preferred by the panelists in color, flavor, taste and sweetness. Sweetness was enhanced with roasted seeds that contain more sugar; whereas taste and flavor was improved with roasting that brings out a characteristic pleasant flavor of hazelnut (Namiki, 2007).

Color is the first characteristic perceived by consumer and the major attribute in food industry. Color of roasted sesame containing yogurts (YRS and YPRS) was preferred due to the pigmentation caused by browning substances released from Maillard reaction that gave better visual appearance to the product. Control yogurt (Y) showed the better score for both texture and acidity. This implies that the high acidity of the supplemented yogurt was perceived by the panelists.

Because no differences were observed, probiotic roasted sesame yogurt (YPRS) and roasted sesame yogurt (YRS) were equally accepted (P>0.05) at first view indicating that probiotic had no effect on sensory attributes of yogurt. The same results were observed when comparing some sensory characteristics (appearance, flavor, texture) between standard and probiotic yogurt made with *L rhamnosus* GR-1 and *L reuteri* RC-14 (Hekmat & Reid, 2006).

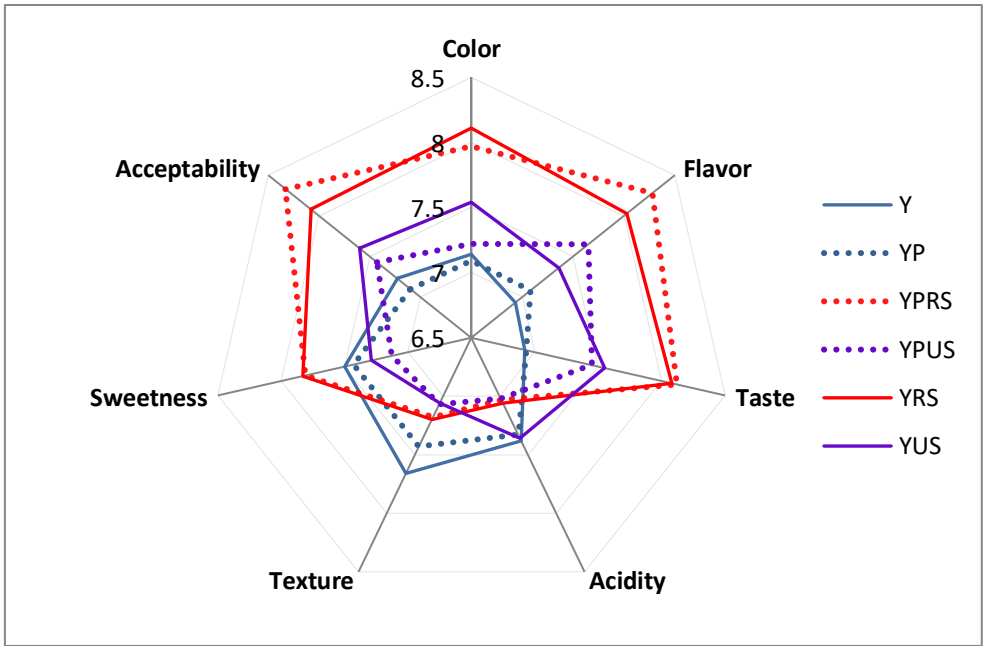


Figure 34: Diagram of sample scores of the sensory analysis

II.3.9. Correlations

Statistical analysis of our results showed strong correlations between yogurt measured parameters (Table XVIII). Microbial viability (yogurt starter culture and probiotics) was moderately associated with TTA and syneresis and highly related to phenolic content, antioxidant and proteolytic activities, for all yogurts.

As expected, pH was inversely related to TTA that in turn was highly associated with syneresis and proteolytic activity ($R = -0.81$ to -0.98, $p < 0.001$).

Post acidification (pH) was generally associated with proteolytic activity, total phenolic content and antioxidant activities of yogurts ($R = -0.78$ to -0.98, $p < 0.05$). Moreover, the pH of yogurts containing sesame seeds (YUS-YRS-YPUR-YPRS) was highly inversely correlated with their titratable acidity and proteolysis, implying that changes in these factors were dependent on the presence of sesame seeds.

The high correlation of sesame yogurts related to antioxidant activity is presumably due to sesame's considerable natural antioxidant compounds (Yoshida et al., 2001). In contrast, probiotic supplementation (YP) nullifies/negates/ attenuates these effects probably due to the

relatively high lactic acid production and reduced acidification attributed to probiotics (*Bifidobacterium* sp and/or *Lactobacillus acidophilus*) (Gomes & Malcata, 1999).

Table XVIII: Correlations between different yogurt parameters during 28 days of cold storing

	CFU	**TTA**	**Syneresis**	**Phenolics**	**DPPH**	**OPA**
pH						
Y	0.939*	-0.543***	-0.543ns	0.891*	0.867*	0.919*
YP	-0.234ns	-0.456ns	-0.697**	0.884*	0.657**	0.213ns
YUS	0.898*	-0.950*	-0.703**	0.731***	0.765*	0.906*
YRS	0.916*	-0.943*	-0.711**	0.669***	0.906*	0.904*
YPRS	0.599**	-0.678**	-0.566**	0.677*	0.861*	0.988**
YPUR	0.593**	-0.8*77	-0.466*	0.544*	0.986**	0.876***

*,**, and *** correlations at $p< 0.0001$, <0.005, and <0.05, respectively, ns, not significant

Conclusion (part II)

This part has focused on (i) evaluating the effect of ground raw and roasted sesame on stirred yogurt quality parameters (pH, titratable acidity, syneresis, proteolytic activity and antioxidant potential), probiotic growth (*Bifidobacterium animalis* ssp. *lactis*, BB-12) and (ii) to determine the acceptability of sesame yogurt by consumers comparing between roasted and unroasted sesame yogurt preparations during 28 days of cold storage (4 °C).

Our results showed that the best concentration of ground sesame was 6% which can be incorporated into yogurt before fermentation under our experimental conditions without whey separation from gel and perturbing lactic bacteria fermentation.

Yogurts enriched with ground raw and roasted sesame seeds manifested the highest probiotic counts of *Bifidobacterium animalis* ssp. *lactis*, BB-12 without affecting starter culture. This better growth of microorganisms in the presence of sesame seeds can be explained by a high intake of carbohydrates (prebiotics) and micronutrients. Titratable acidity, proteolytic activity and radical scavenging activity were improved, whereas pH was reduced as a consequence of organic acid production.

The number of probiotics required in yogurt varies from country to another. It must be greater than 10^6 CFU / g in order to confer health benefits for the consumer. For our yogurts, this number was maintained throughout the storage period. The initial number of bacteria begins to decrease from the day 14. Reduction of prebiotics and micronutrients in yogurt would be the origin of this phenomenon.

It is important to follow and understand the evolution of phytochemicals in yogurt during cold storing to predict the real benefits of their consumption on human health. Total phenolic compounds in sesame yogurts were higher than that of control yogurt. At the end of the storage period we noted quantitative decreases in these compounds in all yogurts. The decreases in phenolic compounds in the yoghurt during storage can be due to their bind with serum proteins such as β-lactoglobulin (β-LG) and α-lactalbumin. These proteins are recognized to have an important affinity with phenolics; interactions are made by hydrogen bonds between the OH of the nucleus aromatic and the carboxyl group of the side chain of the amino acid.

Sensory evaluation of yogurt preparations revealed that yogurts containing roasted sesame (YRS and YPRS) were preferred by the panelists regarding their color, flavor, taste and sweetness. Taste and flavor was improved with roasting that brings out a characteristic pleasant flavor of hazelnut.

The results of this work offer an important alternative for the use of sesame seeds in the formulation of functional foods, *e.g.* yogurt, with beneficial effects on human health.

General Conclusion

General conclusion

The results of this study show that sesame seeds are a rich and potential source of various nutrients: they contain a high quantity of oil (48.43%), as well as 22.38 % protein, 15.41% carbohydrate and 5.67% ash. The oil content was higher than most of the known oilseeds. Chromatographic analysis (GC) of fatty acids revealed that they belong to linoleic-oleic (44.12 - 37.55%) acids. Sesame oil contains 16.24% saturated, 38.94% mono-unsaturated, and 44.52% poly-unsaturated fatty acids.

This study shows that sesame seeds oil is rich in phenolic compounds (lignanes) and sterols as it contains 393 mg/100g of sesamin, 203 mg/100g of sesamolin and 863 mg/100g of total sterols. The most abundant sterol was β-Sitosterol (49.5 %) and Campesterol (33.4%). Only γ-tocopherol was detected among tocopherol homologues in our samples, with 25.93 mg/100 g.

The positive effect of roasting process in culinary preparation was shown in this study. Based on our data, roasting of sesame seeds was helpful for oil extraction yield, generated new antioxidant compounds, and provided better quality and organoleptic attributes.

After roasting, there was no obvious difference in density and refractive index but the iodine, saponification and acid values were affected by temperature. An important primary and secondary lipid oxidation was occurred at T> 250°C, resulting in higher *p*-anisidine value and K232 as well as K268 values. Roasting increased induction period (from 5.51 to 10.50 h), enhanced total phenolic content (from 1522 to 1939 mg/kg) and increased the antiradical activity of extracted oils.

Depending on roasting temperature, a gradual decline was recorded in total levels of phytosterols (maximum of 17.4%), γ-tocopherol (maximum of 10.6%), sesamolin (maximum of 27.5%), sesamin (maximum of 12.5%) and total carotenoids (maximum 65 %). All the investigated oils presented weak triglyceride polymers content, in agreement with the European standards. Regarding the studied parameters, the optimum roasting conditions are suggested to be at 210°C for 20 minutes, to have the better sesame oil with a minimum oxidation and a healthy quality.

A concentration of 6% ground sesame is incorporated into the yogurt containing lactic ferments, with or without probiotics. Yogurt preparations containing ground sesame seeds showed better quality attributes: higher probiotic counts of *Bifidobacterium animalis* ssp. *lactis*, BB-12, increased proteolytic and radical scavenging activities, which were associated with high titratable acidity and TPC as welle as low pH.

After 28 days of storage, the use of the ground seeds helped to maintain the growth of bacterial strains. Raw and roasted sesame selectively impacted probiotic growth with limited effect on yogurt starter culture especially at long cold storage (14-28 days). This confirms its prebiotic effect. The content of phenolic compounds in yogurts decreased during storage. Their complexation with milk proteins could explain this decrease.

Yogurts enriched with roasted sesame has higher sensory acceptability compared to control and probiotic yogurts. Roasted sesame can be successfully incorporated to improve probiotic viability and sensory properties of stirred yogurt, as well as to increase the antioxidant properties.

Our study provides another important use of sesame seeds in probiotic formulation as a basis for the design of functional foods. The results showed that sesame (rich in oil, proteins and lignans) can be incorporated as a new prebiotic in the preparation of probiotic based foods, because it offers a favorable and selective environment for the development of probiotics.

OUTLOOK:

Our analytical data have shown that *Sesamum indicum* seeds represent an important source of lignans, sterols and prebiotics.

These results deserve to be completed, and it would be interesting to:

- Determine the optimum roasting conditions which allow obtaining a product with better nutritional quality with a minimum reduction of bioactive compounds.
- It would be desirable to evaluate *in vitro* and *in vivo* the biological activities of sterols and lignans of sesame oil.

- Evaluate the effect of roasting on the digestibility of seed nutrients

Furthermore, sesame seeds can serve as sources of prebiotics in the formulation of functional foods and nutraceuticals .It would be interesting to:

- Evaluate the antibacterial activity of the yogurt samples.
- Deepen the study of carbohydrates as a growth factor for probiotics and the relationship between this prebiotic potential and the structure of polysaccharides (degree of purity, composition of monosaccharides, degree of polymerization and molecular weight).
- Assess the antioxidant potential of sesame carbohydrates.

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Annexes

Annexes

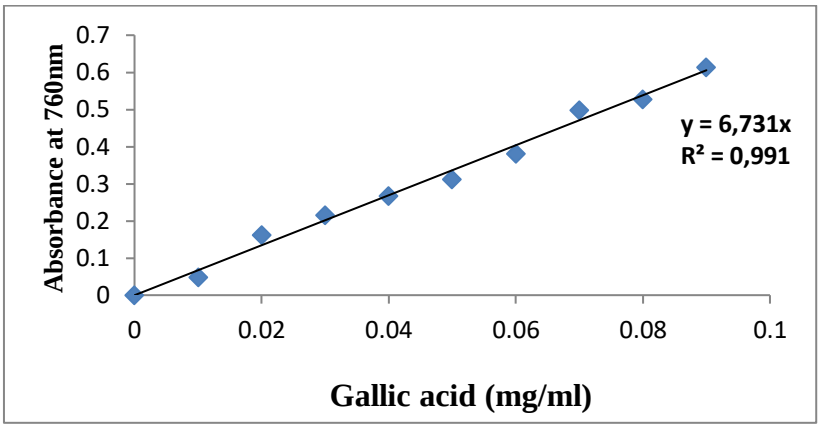

Figure 1: Calibration curve of phenolics

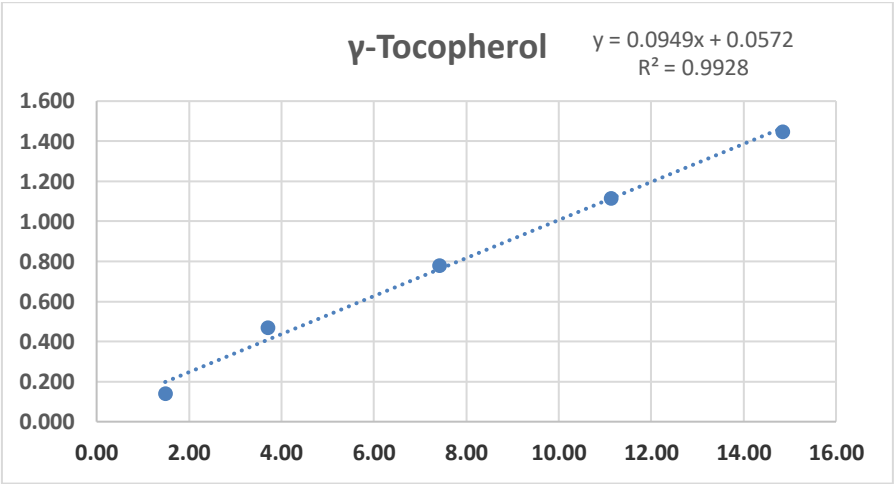

Figure 2: Calibration curve of *γ*-tocopherol

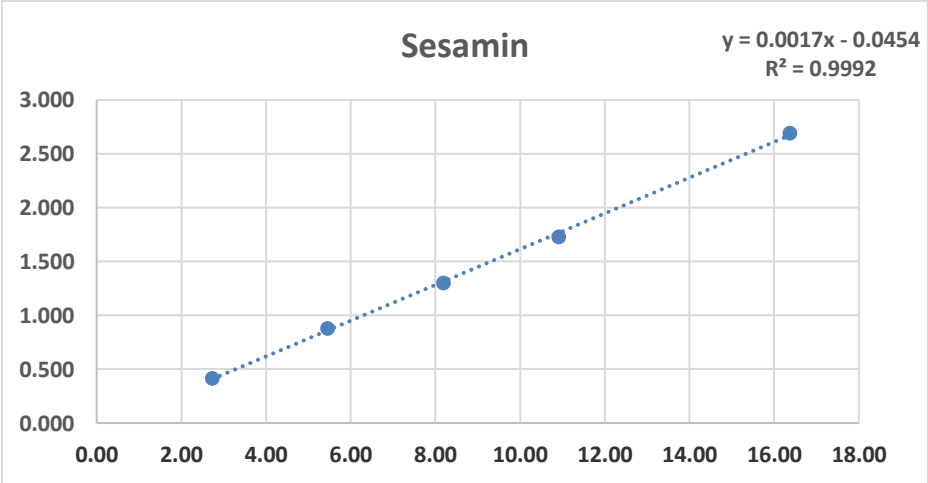

Figure 3: Calibration curve of Sesamin

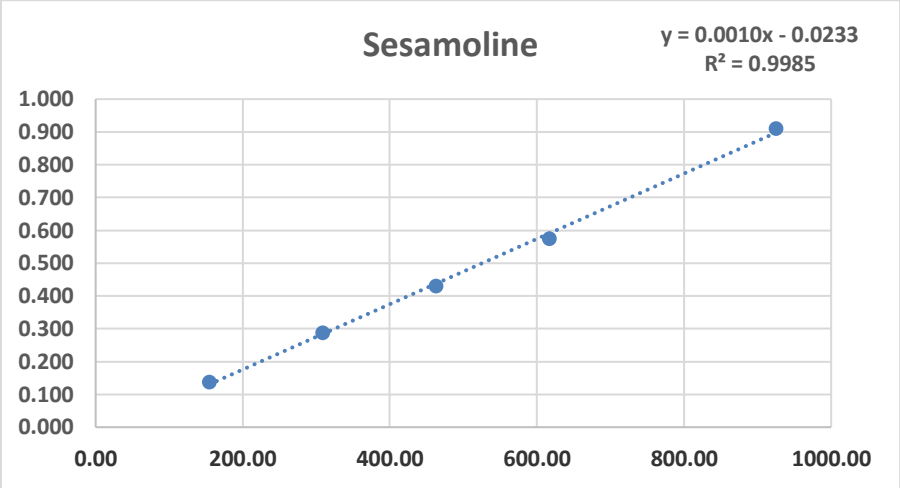

Figure 4: Calibration curve of Sesamolin

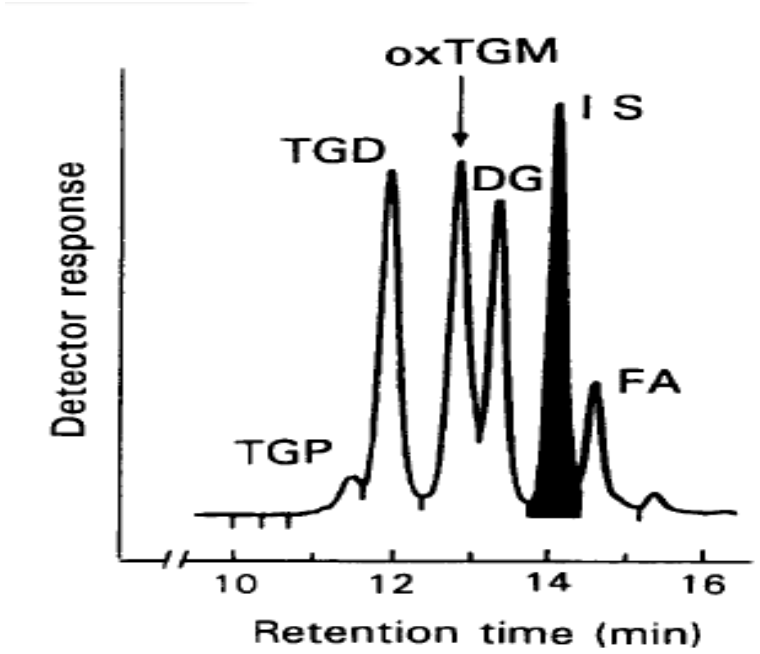

Figure 5: High-performance size exclusion chromatogram of polar compounds, with monostéarine as internal standard (IS). Retention times: 11.5 min triglycerides polymers (TGP); 12 min triglyceride dimmers (TGD); 12,9 min Oxidized triglyceride monomers (ox TGM), 13,4 min diglycerides (DG); 14.1 min IS and 14,6 min fatty acids (FA).

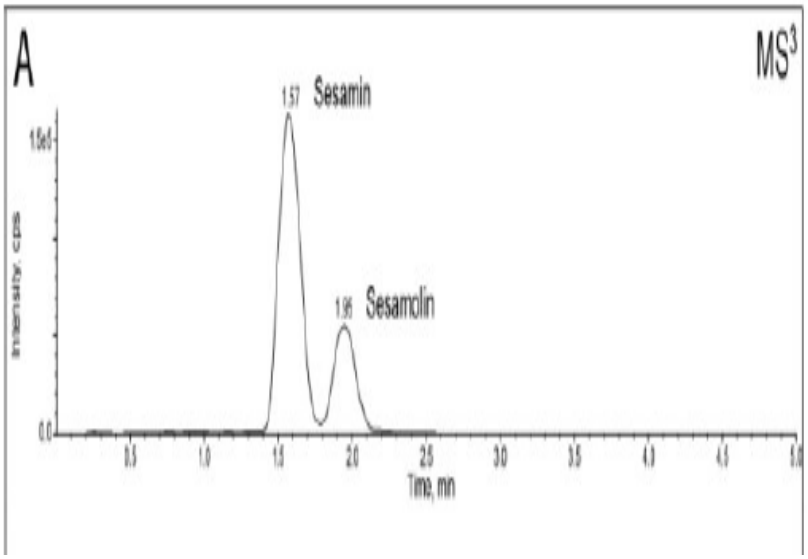

Figure 6: HPLC/ FLD chromatogram of Sesamin and Sesamolin

Preparation of MRS Agar medium

MRS agar	Dissolve 6,2 g of MRS agar in 1000 ml of distilled water, shake on a hot plate, sterilize in autoclave at 120 ° C for 15 minutes
MRS agar au chlorhydrate de cystéine	Dissolve 6.2 g of MRS agar in 1000 ml of distilled water, add 0.5 g of cysteine hydrochloride, stir on a hot plate, sterilize in an autoclave at 120 ° C for 15 minutes.

ABSTRACT

Sesame seed (*sesamum indicum L.*) is one of the world's most important and oldest oilseed crops with a high level content of antioxidant. White sesame seeds were roasted for 20 minutes in an electric oven at different temperatures. Depending on roasting temperature, a small loss was recorded in total proteins carbohydrates and ash, whereas roasting improved oil yield. An important primary and secondary lipid oxidation was occurred at T> 250°C, resulting in higher *p*-anisidine value and K232 as well as K268 values. Roasting increased induction period, enhanced total phenolic content and antiradical activity of the extracted oil. Depending on roasting temperature, a gradual decline was recorded in total levels of phytosterols, γ-tocopherol, sesamolin, sesamin and total carotenoids.. We also investigated the effects of adding raw or roasted sesame seeds on the probiotic viability, quality parameters and consumers acceptability of stirred yogurt. Yogurts containing sesame seeds showed the highest probiotic counts, high titratable acidity, lower pH and highest proteolytic activity and radical scavenging activity. Sesame may have a selective impact on probiotic growth with limited effect on yogurt starter culture especially at long cold storage. Yogurts enriched with roasted sesame were characterized by higher sensory acceptability compared to control and probiotic yogurt. Roasted sesame can be successfully incorporated to improve probiotic viability and sensory properties of stirred yoghurt, as well as to improve the antioxidant properties.

Keywords: *Sesame, oil quality, auxidative stability, probiotic viability, yogurt, lactic fermentation.*

RESUME

Le sésame (Sesamum indicum L.) est l'une des cultures oléagineuses les plus importantes et les plus anciennes au monde. Les graines de sésame blanc ont été torréfiées pendant 20 minutes dans un four électrique à différentes températures. En augmentant la température de torréfaction, une petite perte a été enregistrée dans les protéines totales, les sucres et de cendres, tandis que la torréfaction a amélioré le rendement en huile. Une oxydation lipidique primaire et secondaire importante s'est produite au-delà de 250 ° C, entraînant des valeurs de *p*-anisidine, K232 et K268 plus élevée. La torréfaction a augmenté la période d'induction, amélioré la teneur en phénols totaux et l'activité antiradicalaire de l'huile extraite. En fonction de la température de torréfaction, une baisse progressive a été enregistrée dans les niveaux totaux de phytostérols, de γ-tocophérol, de sésamoline, de sésamine et de caroténoïdes totaux.. Nous avons également étudié les effets de l'ajout de graines de sésame crues ou grillées sur la viabilité de probiotique, les paramètres de qualité et l'acceptabilité des consommateurs de yaourt brassé. Les yaourts contenant du sésame ont montré les taux de probiotiques les plus élevés, une acidité titrable élevée, un pH plus bas, une activité protéolytique et une activité de piégeage des radicaux libres plus importante. Le sésame peut avoir un impact sélectif sur la croissance des probiotiques avec un effet limité sur les ferments, en particulier lors d'une longue conservation au froid. Les yaourts enrichis en sésame torréfié se caractérisent par une acceptabilité plus élevée que les yaourts témoins et probiotiques. Le sésame torréfié peut être incorporé avec succès pour améliorer la viabilité du probiotique et les propriétés sensorielles du yaourt brassé, ainsi que pour améliorer les propriétés antioxydantes.

Mots-clés: *Sésame, qualité de l'huile, stabilité auxidative, viabilité de probiotique, yaourt, fermentation lactique.*

ملخص

بذور السمسم (*sesamum indicum* L) هي واحدة من أهم وأقدم محاصيل البذور الزيتية في العالم حيث تحتوي على نسبة عالية من مضادات الأكسدة. تم تحميص بذور السمسم الأبيض لمدة 20 دقيقة في فرن كهربائي بدرجات حرارة مختلفة. اعتمادًا على درجة حرارة التحميص ، تم تسجيل خسارة صغيرة في الكربوهيدرات الكلية والبروتينات والرماد ، في حين أدى التحميص إلى تحسين محصول الزيت. حدث أكسدة دهنية أولية وثانوية مهمة عند T> 250 درجة مئوية ، مما أدى إلى ارتفاع قيمة p-anisidine و K232 وكذلك K268. زيادة فترة التحميص ، وزيادة المحتوى الفينولي الكلي والنشاط المضاد للفطريات في الزيت المستخلص. اعتمادًا على درجة حرارة التحميص ، تم تسجيل انخفاض تدريجي في المستويات الإجمالية للفيتوستيرول ، بيتا توكوفيرول ، سيسامولين ، سيسامين والكاروتينات الكلية .. لقد بحثنا أيضًا في تأثيرات إضافة بذور السمسم الخام أو المحمص على جدوى البروبيوتيك ومعايير الجودة ومقبولية المستهلكين من اللبن الزبادي. أظهر الزبادي المحتوي على بذور السمسم أعلى عدد من الكائنات الحية المجهرية ، وحموضة عالية قابلة للمعايرة ، وانخفاض درجة الحموضة ، وأعلى نشاط تحلل للبروتين ونشاط إزالة جذري. قد يكون للسمسم تأثير انتقائي على نمو الكائنات الحية المجهرية مع تأثير محدود على زراعة بداية الزبادي خاصة في التخزين البارد الطويل. يتميز الزبادي المخصب بالسمسم المحمص بقبول حسي أعلى مقارنة باللبن الزبادي المعزز بالنمو. يمكن دمج السمسم المحمص بنجاح لتحسين جدوى البروبيوتيك والخصائص الحسية للزبادي المخفوق ، وكذلك لتحسين خصائص مضادات الأكسدة.

الكلمات المفتاحية: السمسم ، جودة الزيت ، حيوية البروبيوتيك ، الزبادي ، التخمير اللبني.