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MASTER

Thème

L'industrie laitière et l'innovation en Algérie, yaourt à valeur ajoutée

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Dedication

I would like to dedicate this modest work to the people dearest to my heart;

My mother DAHBIA and the memory of my father SALEM that ALLAH welcomes him in his vast

Paradise.

To my dear sisters KARIMA, FATIMA, NOURA, WAZNA and my two brothers KAMEL and

AZZEDINE

My dear cousins MAALAZ, NACIRA, LAMINE, KAMEL, SAMIR AND SOFIANE

To my grandmother and grandfather

Also, to all my friends especially SARAH, KENZA,TAOUS AND DYHIA

To my dear partner Nedjma and all her family, for the sister she was

And that she will remain for me, for all the wonderful moments since

Our acquaintance.

To the entire class of PTL 2022

Boussaid Souaad

Dedication

I dedicate this modest work…

To my father

My highest example and my model of perseverance for always going forward and never giving up and for his continuous teaching;

To my mother

For her affection, her patience, her understanding, her availability, her constant listening and her support;

To my sister: Zina, for her constant encouragement and moral support;

To my brothers: Karim, Samir, Aziz and Reda

To my dear ones: Islam, Adel, Adem, Ilyan; Ritaj and Anis.

To my dear and best friends: Sylia, Hakima, Nabila, Hania, Laldja, Tamazight, Dalila and Nassima;

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To my Faithful friends, colleagues, cousins who are dear to me.

Djeraoune Taidir

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Abed Rrahman Mira of Bejaia

List of abbreviation

g.	Gramm
UHT.	Ultra higt temperature
°C.	Celsius degree
PDO.	Protected Designation of Origin
PH.	Hydrogen potential
UBD.	Use by date
SC.	Sour cream
OEO.	Orange essential oil
AFNOR.	French national organization for standardization
BHA.	Butylated hydroxyanisole.
BHT.	Butylated hydroxytoluene.
mM.	Milli mole
ml.	Milli liters
µl.	Micro liters
AC.	Absorbance control
AS.	Absorbance sample
FATM.	Flore aerobic mesophilic flora
YGC.	Yeast Glucose Chloramphenicol
L.	liters
PCA.	Plate Count Agar
VRBG.	Violet Red Bile Glucose Agar
VRBL.	Lactose bile medium with crystal violet and neutral red
NaOH.	Sodium hydroxide.
V.	Volume
D°.	Dornic degree
ISO.	Inter national organization for standardization
Rpm.	Rotation per minute
FCU.	Forming Colony Unit
FAO.	Food and Agriculture Organization
H^+.	The hydrogen cation
J.O.R.A.	jornal official republic Algerian

List of appendix

List of figure

List of tables

Summary

Table of contents

Experimental part

Results and discussions

Introduction:

The conservation of dairy products constitutes a major precaution from their production to their consumption. Their alterations can lead to health and commercial risks, following their contamination by the microorganisms which develop in the product **(FAO, 1995).** Sour cream is a dairy product relatively rich in fat (MG), which makes it susceptible toalterations by microorganisms capable of hydrolyzing fat, causing bitterness and rancidity to the product **(Jamotte, 1967; Fredot, 2005).**

Consumers are demanding partial or complete substitution of synthesized preservatives due to Their adverse health effects. This fact has led to an increasing interest in developing more natural alternatives in order to enhance the shelf life and safety of food **(Bukar et al., 2010; Arora et al., 2013**). Yeast and moulds can grow on the surface of the product in a package that is not airtight. In the event of extended storage, the enzymes of lactic acid bacteria can hydrolyze the lactoglobulin and cause bitterness in the product **(Tamime, 2006).**

Methods of natural protection began to be popular in food attribution and new preservation techniques focused on the use of plants which have antioxidants and antimicrobial effect. Indeed, studies have demonstrated that diets rich in antioxidants (phenolic compounds, essential oils) have many health benefits and confer longer life expectancy. Bioactive substances are mainly present in leavesand seeds of some plants and have been implicated in preventing food from the free radical mechanism.**(Anwar et al., 2007).**

Essential oils are known for their antioxidant and antibacterial effects, they have long been used in medicine, cosmetics, and herbal medicine. Essential oils have been used in the food industry to enhance the taste of foods and are being studied to better understand their effectiveness as natural preservatives. These natural agents reduce or replace chemical or synthetic preservatives that have adverse health effects (**Bessah and Benyoussef, 2015**).

It is in this context that our study takes place, the main objective is the possibility and the impact of the use of orange essential oil as a preservative in sour cream.

Our work is divided into three parts:

- The first part is devoted to a bibliographical synthesis on cream and orange essential oil;

- In the second part, we adopted an experimental approach that relates to the description of materials and methods used,
- Finally, the third part detailed analysis of the results and their discussion followed by a general conclusion and perspectives.

Bibliographical part

I. Overview of cream:

I.1.Definitions:

I.1.1. Cream:

Cream is a fluid dairy product with varying degrees of fat content in the form of a fat-un skimmed milk emulsion obtained by physically separating it from milk **(Codex Alimentarius, 2003).**

Cream can be defined as an emulsion of dairy origin of the type fat in water, i.e. the fat particles are dispersed in droplets in the water phase **(Vilain, 2010).** The term 'cream' is reserved for products with a fat content of 30% or more. The texture of dairy cream varies according to the lactic ferments added, the addition of authorized additives and the fat content **(Merigaud et al., 2010).**

I.2. Denominations:

I.2.1. Raw cream:

It is the cream obtained just after skimming that has not undergone any particular heat treatment, its consistency is liquid and its flavor is sweet **(Vignola et al., 2002).**

I.2.2. Sour cream:

It is rich in fats, obtained by the fermentation of cream by bacteria producing lactic acid. It is widely used in the USA, Eastern and Central Europe and Europe as well as in English-speaking countries. By definition, sour cream or cream sour in Quebec is a pasteurized cream product that is acidified by bacteria producing lactic acid. It must contain at least 18% fat and at least 0.5% lactic acid **(FDA, 2008).** It is a popular fermented milk product that comes back under different names and slightly different forms slightly different forms worldwide. This variation in form is mainly due to the fat content and also the acidity and/or viscosity of the finished product.

In addition, the end use of the product or their application in food varies from country to country. In the USA sour cream is used as a tasty topping, and can also be found as an ingredient in cakes, cookies, and/or as a key ingredient in various hot in various hot foods, while in France, sour cream is generally used as a topping for a garnish for fruits, and for salads **(Meunier-Goddik, 2004).**

I.2.2.1.Composition of sour cream:

The essential contributions of sour cream are lipids and vitamin A. It also provides an interesting amount of calcium and vitamin D **(Jeantetc et al., 2008) table 01.** The amount of lipids in cream can impact its nutritional value. For example, greater fat in the cream means less lactose, minerals, and proteins. When compared to milk, it provides more of these nutrients if it has higher vitamin A and carotene. Thick cream contains aldehydes and ketones, which give it its special taste, as well as lactic acid (about 8g/l) **(FAO, 2010).**

Figure.01: average composition of fresh cream with 30 % fat **(Jeantet et al., 2008)**

Composition	Content (in %)
Water	59
Fat content	30
Lactose	3.1
Protein	2.3
Mineral	0.5
Calcium	$90mg.100^{-1}g^{-1}$

Milk lipids are secreted in the form of colloidal assemblies called milk fat globules. Technological processes used in the dairy industry, e.g. thermal and mechanical treatment, can affect the structure and composition of milk components and alter their technological and nutritional properties **(Christelle et al., 2015).**

I.2.3. Fresh pasteurized cream liquid:

It has not undergone seeding or ripening, it therefore retains its fluid and soft texture but it is quite texture but it is quite fragile. This cream is rarely marketed except for restaurant owners under the name "crème fleurette" but this name is generic and not legal. It is highly appreciated for its ability to expand, i.e. to be beaten to integrate to be beaten to integrate the air which makes it light and voluminous until the stage of whipped cream **(Fredot, 2005).**

I.2.4. Fresh pasteurized cream thick (or matured):

Following pasteurization, if we want a thick cream, we proceed to the maturation. The process consists of cooling the cream to "crystallize" part of the fat (physical ripening) and then to inoculate it with lactic ferments taken from creams, particularly from particularly aromatic creams (biological ripening) with a high level of acidity **(Fredot, 2005).**

I.2.5. Fermented cream:

Fermented cream is the dairy product obtained by the fermentation of cream, reconstituted or recombined cream, by the action of appropriate microorganisms, which leads to the reduction of the ph with or without coagulation. When the content of specific microorganisms is indicated, directly or indirectly, in the labeling or through nutritional claims during the commercial process, these microorganisms must be present, viable, active, and abundant in the product at the date of minimum durability. If the product undergoes heat treatment after fermentation, the requirement for the viability of the microorganisms no longer applies **CODEX STAN 288-1976.**

I.2.6. UHT cream

The UHT treatment of creams is an increasingly common practice in the industry because of their prolonged conservation, appropriate for a more expensive product. The organoleptic, nutritional and functional qualities are preserved. The packaging is done aseptically **(Pouliot et al., 2010).**

I.2.7. Sterilized cream

Once packaged, the raw cream is sterilized at 115 ° C for 15 to 20 minutes, and then cooled. This process develops a taste of cooked or caramel **(Merigaud et al., 2009).**

I.2.8. Whipped cream

This name is reserved for whipped cream that contains at least 30g of fat for 100g.The only additives are sucrose and any natural flavourings **(Vignola et al., 2002).**

I.2.9. Light cream

At the skimming, we seek to obtain a fat content slightly higher or equal to that of the finished product, because equal to that of the finished product, because the emulsion in a cream rich in fat (45% or more) is less stable. Cold skimming preserves the viscosity of the cream, while hot skimming should be followed as soon as possible by pasteurization to limit the action of lipases activated by the temperature and agitation. For standardization, whole milk is preferable to skim milk because it can better prevent the separation of cream from serum **(Pouliot et al., 2010).**

I.3. Manufacture of cream for consumption:

Consumer creams are distinguished according to:

- Their fat content: from 15% for low-fat creams to over 35% for AOC creams.
- The heat stabilization treatment: pasteurization, sterilization, UHT sterilization, freezing, deep-freezing;
- The functionalities expected by the consumer: liquid, thick Sweetened, flavored, to be whipped, packaged in spray cans (metal packaging under pressure Packaging)... etc.

Consequently, they call upon different operations and treatments in order to achieve the set Objectives (**Boutonnier, 2007).**

I.3.1. Skimming

The milk is heated to 50 ° C followed by a separation of the fat of milk during this operation, Skimming operation giving two products: skim milk and cream. This separation is this separation is done by centrifugation with sophisticated machines at a temperature of 35°C **(Boutonnier, 2007).**

I.3.2. Pasteurization

Pasteurization consists in a thermal treatment at high temperature which is done between 85°C and 90°C for 15 to 20 seconds while preserving the organ oleptic qualities of the cream. It causes the destruction of pathogenic germs and germs and most saprophytic germs, the destruction of lipases that cause rancidity, the formation of sulphurous compounds which oppose the oxidation of lipids, and the subsequent control of the lactic maturation of the cream **(Fredot, 2005).**

I.3.3. Maturation:

Pasteurized creams can be matured in the presence of mesophilic lactic acid bacteria, namely Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris, Leuconostoc mesenteroides subsp. Cremoris and Lactobacillus acidophilus **(Jeantet et al., 2006b; Jeantet et al., 2008; Hoffmann, 2011),** acidifying, aromatic and sometimes thickening agents used at 0.5% **(Fredot, 2005; Jeantet et al., 2008).** Maturation makes the cream more aromatic by the conversion of citrate into diacetyl by Leuconostoc, and gives it better protection by production of lactic acid and bacteriocins (nisin, diplococcin) **(Jeantet et al., 2008)** . The maturation phase takes place for a period of 12 to 18 hours at a temperature between 12 and 22°C **(Fredot, 2005; Jeantet et al., 2006b).** The acidification leads to the progressive destabilization of the casein micelles which, in association with the homogenized fat globules, lead to a thickening of the cream. The most significant modifications, including the increase in viscosity, appear for pH values below 5 **(Jeantet et al., 2006b).**

I.3.4. Cooling and Packaging:

After maturation, the cream is cooled and packaged, then stored in cold storage (6°C) and marketed **(Fredot, 2005)**.

- ✓ The method of packaging and the type of packaging used varies according to the product.
- ✓ The cream is distributed in jars on a filling machine equipped with piston dozers.
- ✓ The jars are then labeled and stored in a cold room **(Dudez et al., 2002).**

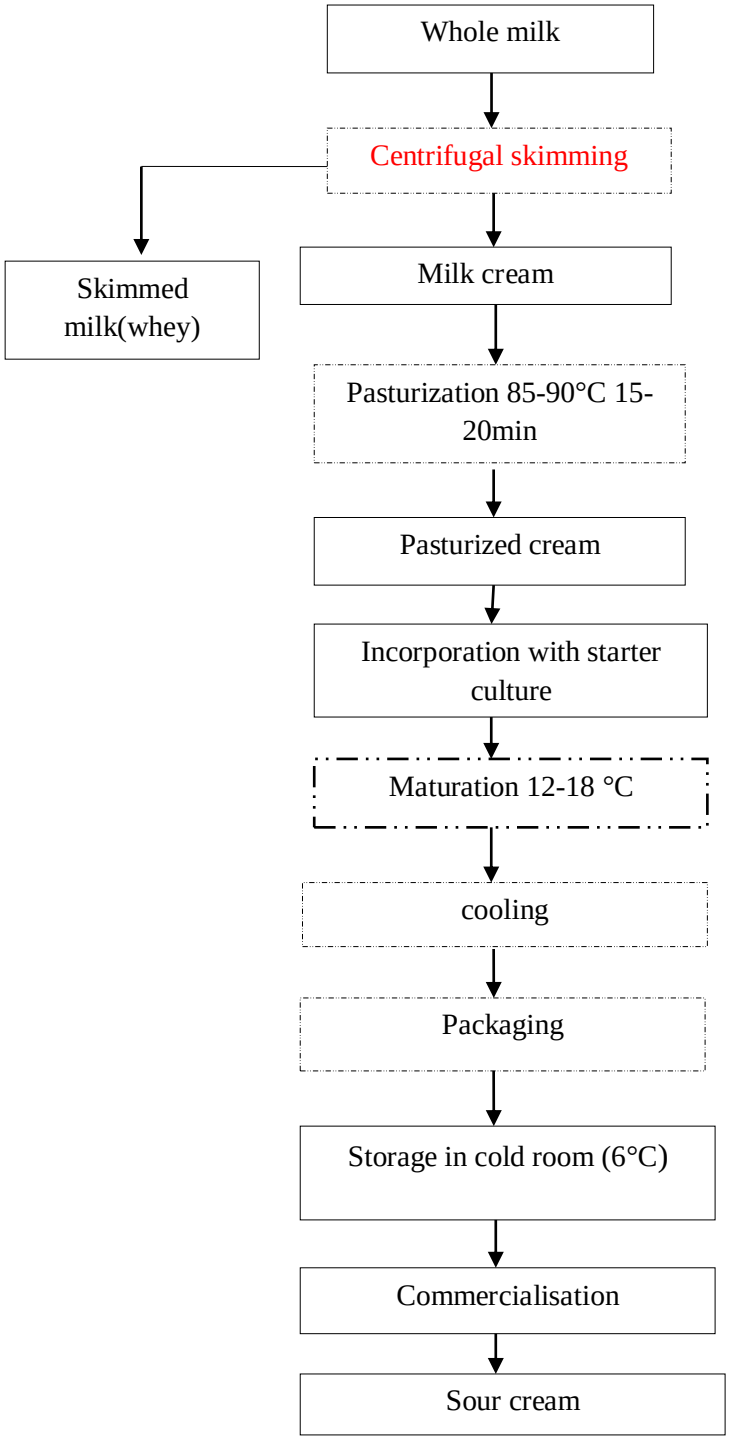

Figure.01: Process flow chart of a typical sour cream process **(Fredot, 2005)**

I.4. Storage of different types of cream:

The use-by date of the different creams is variable **(table 2)**, depending on the microbial load following the heat treatment applied and also depending on the addition of lactic ferments which play an important role in the preservation of dairy products.

Table.02: Storage of different types of cream **(Fredot, 2005)**

Types of cream	UBD	Storage (before opening)	Conservation
Raw cream	**7 Days**	**4-6°C**	**4-6 °C**
Liquid cream fresh	**15 Days**		
Thick cream fresh	**30 Days**		
Sterilized cream	**3 month**		
UHT sterilized cream	**8 month**		

I.5. Alteration of sour cream:

Fresh cream is a food product that contains several nutrients such as proteins, carbohydrates, fat-soluble vitamins and especially fat in addition to its content high in water (59%), which makes it subject to different types of alterations: microbial, enzymatic and physicochemical **(Everett, 2007).**

I.5.1. Microbial alteration:

Cream can contain all the germs encountered in milk; even it can carry the pathogenic bacteria **(Guiraud, 2003).** Several types of microorganisms can be agents of degradation. First of all, the lactic acid bacteria can cause very strong acidity. Coliforms and Enterobacteriaceae can cause bad taste. Lipolytic bacteria destroy and oxidize matter oily, causing the cream to go rancid. Proteolytic bacteria can degrade caseins. In the end the yeasts and molds can cause taste alterations (musty, pungent, malty, and caramelized) **(Guiraud, 2003).** An alteration of the hygienic quality of fresh cream jeopardizes the health of the consumer, this alteration is generally invisible, it is due to a development of pathogenic microorganisms responsible for food poisoning of varying severity. These germs pathogens release toxins .which make the cream unsafe to consume even if the responsible microorganism is no longer alive in the product. Some microorganisms themselves present a danger to the consumer; this is the case of enterobacteriaceae which produce endotoxins **(Bouix and Leveau, 1984).**. Other pathogenic microorganisms may be found in milk and dairy products, including Yersinia enterocolitica, Campylobacter jejuni, Coxiella burnetii, Streptococcus agalactiae, Clostridium botulinum, Bacillus cereus, molds toxins and viruses. The presence and persistence of these germs in milk and dairy products depend on their resistance to the pasteurization treatment that the milk can undergo raw milk and the initial level of contamination in raw milk **(Brisabois et al., 1997).** An alteration in the marketable quality of the cream modifies its plastic characteristics and organoleptic (rancidity, taste alteration), this alteration, although not dangerous for the consumer, renders the cream unmarketable. The failure of the technology implemented work for the physiological stability of the product is the main cause of this alteration (which generally produced slowly during storage of the cream) **(Bouix and Leveau, 1984).**

I.5.2. Enzymatic alteration:

I.5.2.1.Proteolysis:

Proteolysis is an enzymatic degradation of proteins by proteases. These last ones are enzymes that catalyze the hydrolysis of peptide bonds of proteins and produce protoses, peptones, peptides or even amino acids depending on the degree of hydrolysis **(Vignola, 2002).** In milk and milk products, proteolysis has two main origins: bacterial or native **(Haddadi, 2006).** The two main milk proteases are plasmin and Lysosyme that has antibacterial properties and plasmin plays an important role in milk, as it hydrolyzes caseins, which releases peptides of different lengths. The hydrolysis of caseins leads to the formation of very short hydrophobic peptides, which confer bitterness to the cream **(Goursaud, 1985 and Moller, 2000).**

I.5.2.2.Lipolysis:

Lipolysis is an enzymatic reaction of degradation of fat that results in an increase in free fatty acids In milk by an increase in the content of free fatty acids **(Kuzdzal-Savoie, 1982,**

Chilliard and Lamberet, 1984 In Heuchel et al., 2003).

Lipolysis is due, most often, to the action of enzymes present in the dairy products themselves. These are the natural lipases of milk that degrade the fat. In the final stage of their action, they Break down the glycerides into glycerol and fatty acids. The degradation is often partial with the production of intermediate mono and diglycerides. The "rancid" defect appears when fatty acids are released (butyric acid). The lipolysis of creams is marked by modifications of the surface tension and a certain braking of lactic acidification **(Jamotte, 1967).**

The natural lipase is very unstable and highly sensitive to heat, it is inactivated by heating for 30 min at 55°C to 75% **(Goursaud, 1985).** These lipases are able to act at low temperature and are thermoresistant contrary to the germs that produce them produce them **(Demazeaud, 1997).**

Other enzymes may be involved in the degradation of milk fat, in particular lipases secreted by yeasts and molds **(Weber, 1994).**

I.5.3. Lipid oxidation:

Oxidation of fat is probably the chemical transformation, causing the most important problem in dairy technology. The oxidation of the various fat constituents can lead to the appearance of many tastes such as oxidized or metallic taste. Phospholipids are most exposed to lipid oxidation given their chemical structure and their location on the periphery of fat globules **(Vignola, 2002)**. The oxidation of fat occurs mainly on the chains of unsaturated fatty acids and this in three steps. First, there is formation of a radical in α position of a double bond, **i.e.** on the carbon atoms adjacent to the carbon atoms carbon that form the double bond. This radical is initiated by light or certain

metals such as nickel and iron **(Vignola, 2002; Jeantet et al., 2006a; Jeantet et al., 2008).** Subsequently, oxygen adds to the radical and causes the accumulation of fatty acid hydroperoxides. Finally, these last ones decompose into different products such as aldehydes, alcohols, ketones and free fatty acids **(Vignola, 2002; Jeantet et al., 2006a).** The means implemented to prevent this decomposition consist in protecting the milk and dairy products from light, in particular through the use of opaque packaging, and against any copper and iron contamination. In addition, natural antioxidants are very effective in preventing fat oxidation **(Vignola, 2002).**

II. Overview on essential oil:

Essential oils (EO's) are volatile hydrophobic liquids extracted from plants which are often rich in aroma. They are a mixture of secondary metabolites, often terpenoids that play an important role in plant defense system and possess strong anti-microbial activities. **(Bhavaniramya et al, 2019)**

The French standardization association **(AFNOR, 2000)** defines an essential oil as being a product obtained from vegetable matter, either by steam distillation, or by mechanical processes, or by dry distillation. The essential oil is then separated from the aqueous phase by physical processes.

II.1.Orange essential oil:

Orange oil is an essential oil produced by cells within the rind of an orange fruit (citrus saneness fruit) .In contrast to most essential oils ,it is extracted as a by – product of orange juice production by centrifugation , producing a cold-processed oil. it is composed of mostly (greater than90%) d-limonene. D-limonene can be extracted from the oil by distillation **(Bousbia, 2004).**

II.2.Location and biosynthesis of essential oils:

According to **Bardeau (2009),** most plants contain essences; however, they are particularly abundant in aromatic plants of the following families: labiatae, umbelliferae, myrtaceae, rutaceae, lauraceae, conifers, terebinthaceae. Depending on the case, essential oils are extracted from flowering tops or flowers (chamomile), leaves (mint), seeds or fruits, roots, bark (cinnamon), zest (grapefruit) or wood (cedar).

Essential oils are found in specific secretory cells. These are specialized histological structures which are used for their synthesis and storage. These cells are rarely in an isolated state, but most often grouped together in pockets, in secretory ducts or in secretory hairs. They are most often at the periphery of the external organs of the plant **(Kaloustian and Hadji-Minaglou, 2013).**

II.3.Chemical composition of orange essential oil:

Main chemical compound: Monoterpenes (90 to 95%) (Limonene) Other chemical compounds: Monoterpenols (2 to 6%) (Carvone), Terpene aldehydes, Coumarins and Furocoumarins Citrus .essential oils are mixtures with over 200 compounds that can be grouped into non-volatile (1-15%)

and volatile (85-99%) fractions. This last fraction contains mainly monoterpenes and sesquiterpenes as well as a small amount of oxygenated monoterpenes **(Mondello et al, 2005).** D-Limonene is the main constituent of fruits and essential oils obtained by expression or not distillation of fruit zest belonging to the genus Citrus **(Kaloustian and Hadji-Minaglou, 2013).** For example: The oil extracted from the peel of grapefruit can contain 90% limonene **(Morton, 1987).**

II.4.Biological activities of essential oils:

EOs possess a variety of well-known therapeutic properties such as antiseptic, anti-inflammatory, diuretic, tonic, and antispasmodic activity **(Worwood, 2016).** Many essential oils and their volatile components have been found to have strong antibacterial activity, which inhibit the growth of some bacteria, fungi, and other microorganisms, and suppress the production or accumulation of mycotoxins **(Bluma, Amaiden, Daghero, & Etcheverry, 2008; Moumni et al., 2020).** The antibacterial activity of essential oils is mainly caused by its complex active components (mainly including alcohols, aldehydes, ketones, and phenols) **(Li, Cai, & Liu, 2019; Yang et al., 2011).** Essential oils can use its own hydrophobicity to penetrate lipids, thus destroying the structure of the cell wall and changing the permeability of the cell membrane: this leads to the outflow of ions and matter within the cyst, causing cell death **(Cosentino et al., 1999**; **Dorman & Deans, 2010).**

II.4.1. Antioxidant activity of essential oil:

The application of antioxidants plays an important role in inhibiting oxidative reactions in various products; in addition, these could prevent diseases related to the oxidative stress in the human body **(Liu et al., 2012).** Application of synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), has resulted in the appearance of significant side effects, which explains the growing interest in search for natural antioxidants **(Olmedo, Nepote, & Grosso, 2014).** EOs is added to edible preparations either by mixing them into the product or in active packaging as an edible coating. This is a suitable alternative for prevention of autoxidation and prolongation of shelf life. Analyzing the antioxidant potential of essential oils is a crucial aspect for the food industry since common test are inappropriate for them and may yield contradictory results **(Loizzo et al., 2009).**

II.6.Orange essential oil uses:

The essential oils marketed in the world are intended for four major industrial sectors: cosmetic perfumery; technical perfumery (soaps, detergents); food and medicine (alternative medicine and pharmaceuticals) **(Grysole, 2005).** The food industry uses essential oils to enhance the taste, flavor, and color of food **(Pingot, 1998; Bruneton, 1999; Grysole, 2005; Aprotosoaie et al., 2010).** The soft drink sector is a major user of essential oils **(Grysole, 2005).**EOshave different chemical

composition profiles, are used as natural food preservatives. This use is due to the presence of compounds with antimicrobial and antioxidant properties **(Conner, 1993; Hammer et al., 1999).** The most used essential oil in the world is of orange **(Grysole, 2005).**

Table.03: the application of orange essential oil on dairy products

Essential oil	**Dairy products**	**Remarks**	**references**
Orange	**Milk**	flavoring agent because of its volatile Components. prevent the spoilage of food from microbes in several food matrices. Natural and effective antimicrobial And antioxidant agent.	**(Licon et al. 2020).** **(Jemaa et al. 2017)**
	Yogurt	no significant difference in pH	**(Azizkhani and Tooryan 2016)**
	Cheese	growth restriction and reduction in microorganism survival	**(Gouvea and al, 2017)**
	Ice-cream	Having the lowest total aerobic mesophilic and psychrophilic bacteria, yeast and mold. Apart from this total coliform group bacteria *Salmonella*, *E. coli*, *Listeria* and *S. aureus* Were absent. Another advantage was the use of natural additives as the essential oils can be added for their antimicrobial, preservative and flavoring properties	**(Al-Rimawi and al 2019)**

Experimental part

II. Materials and methods:

Our work consists initially of the enrichment of sour cream with Orange EO in the sour cream manufactured at the "DANONE Djurdjura" (Akbou). The Protocol followed in our study is described in the following flow chart:

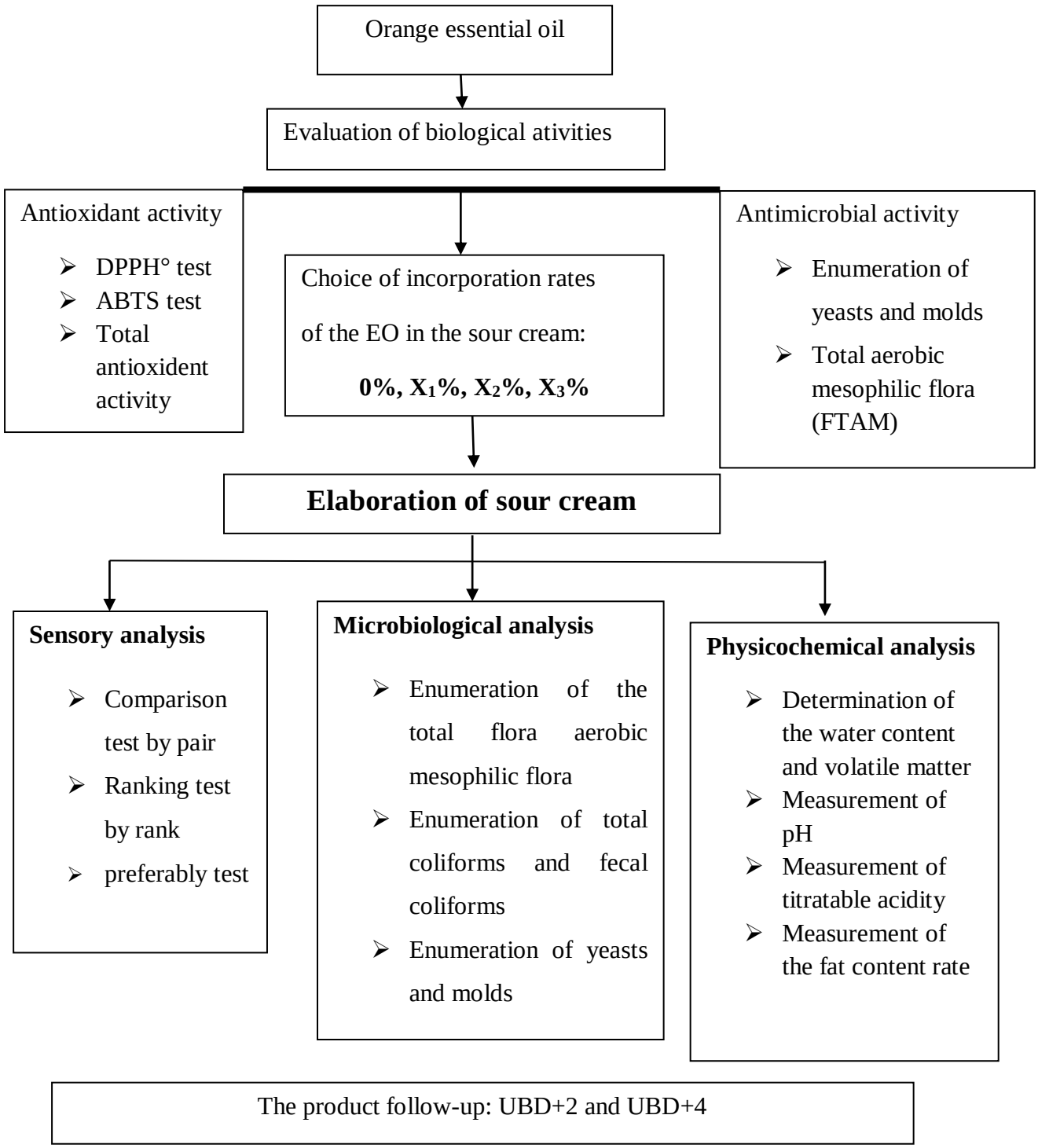

Figure 02: Flow chart of the study methodology

I. Evaluation of the biological activities of the essential oil of OEO:

I.1. Antioxidant activity:

The antioxidant activity of the essential oil was evaluated by the method of measuring the power of scavenging radical DPPH.

I.1.1. DPPH test:

- **Principle:**

DPPH (2,2 diphenyl-1-picryl hydrazyl) is a stable radical that has a single electron on the nitrogen atom, characterized by a violet color and a maximum spectral absorption peak at 517nm(**Alam,bristi et al 2013).** In the presence of antioxidant the single electron becomes paired, leading to the decoloration of DPPH from purple (radical form DPPH·) to yellow (reduced form DPPH-H) .This discoloration thus represents the sample capacity to trap this radical. **(Morikawa T et al., 2004)**.

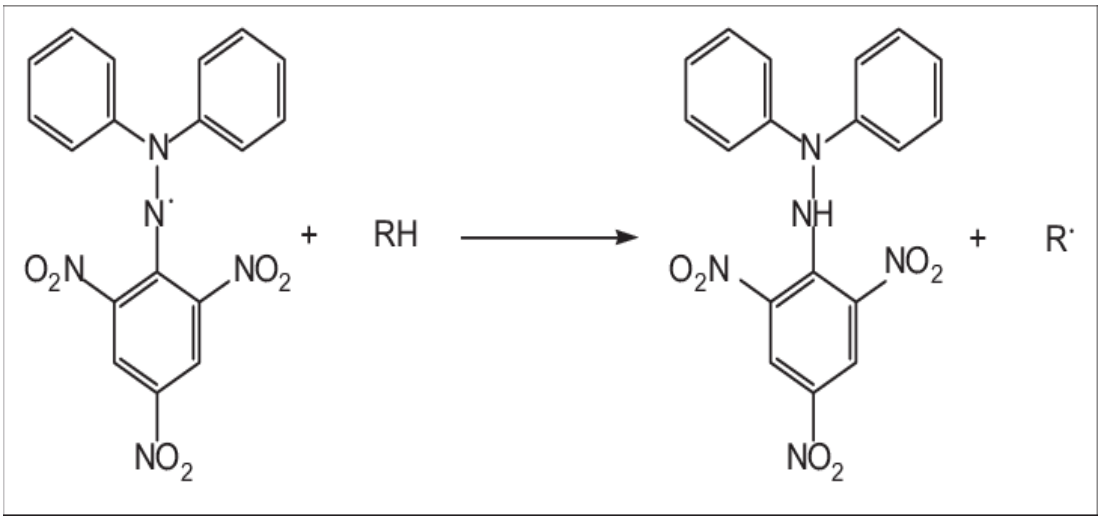

Figure 3: Structure of DPPH and mechanism of its reduction by an antioxidant (**Morikawa T et al., 2004)**

- **Procedure:**

1 ml of extract was added to 2ml of DPPH (2.10^{-4}M/L in methanol) and the mixture was left in the dark at room temperature for 20minfollowed by reading the absorbance at 517 nm.**(Achat et al 2012) .**

- **Reading:**

The free radical scavenging activity of each solution was then calculated as percent inhibition according to the following formula:

$$\%\text{Inhibition} = \frac{Ac - As}{Ac} \times 100$$

Ac: the absorbance control experiment.

As: the absorbance sample.

I.1.2. ABTS test:

The method that determines the scavenger activity of the ABTS radical is based on the ability of an antioxidant to trap the greenish blue staining of the ABTS • + (2. 2'-azinobisethylbenzothiazoline-6-sulfonic) cationic radical. Transforming it into colorless ABTS-H +, by a hydrogen donation **(Antolovich, Prenzler et al. 2002).**The decrease in absorbance caused by the antioxidant reflects the capture capacity ofthe free radical.The percentage inhibition of the ABTS • + radical is evaluated by the method of **(Pellegrini et al. 1999),** which is based on the ability of antioxidants to interact with theradical ABTS • +, reducing its absorbance at 734 nm.

- **Procedure:**

A free radical solution (7 mM ABTSand 2.45 mM potassium per sulfate) wasprepared and incubated in the dark at roomtemperature for 12-16 h before use.This solution was then diluted with ethanol to anabsorbance of 0.705 ± 0.02 at 734 nm. Control, extractsamples were prepared, respectively; 2 ml of radical solution, mixed with 20 µl of extraction solvent Absorbance was read at 734 nm after 6 min of incubation at room temperature in the dark.

- **Reading :**

Antioxidant activity was calculated according to the equation:

The percentage of the ABTS was calculated using the following formula:

$$\text{I\% of ABTS} = \left(\frac{A\,c - A\,s}{A\,c}\right) \times 100$$

Ac: absorbance control

Ac: absorbance sample

I.1.3 Total antioxidant capacity:

The total antioxidant capacity (TAC) wasevaluated as described by **Prieto et al. (1999).**A volume of 200µl extract was added to 2ml of reagent (0.6M sulfuric acid, 28Mm sodium phosphate, and 4Mm ammonium molybdate) the mixture was incubated at 95°C for 90 min Afterthe samples had been cooled, the absorbance wasmeasured at 695 nm. The results were expressed asmg ascorbic acid equivalent per g of dry matter.

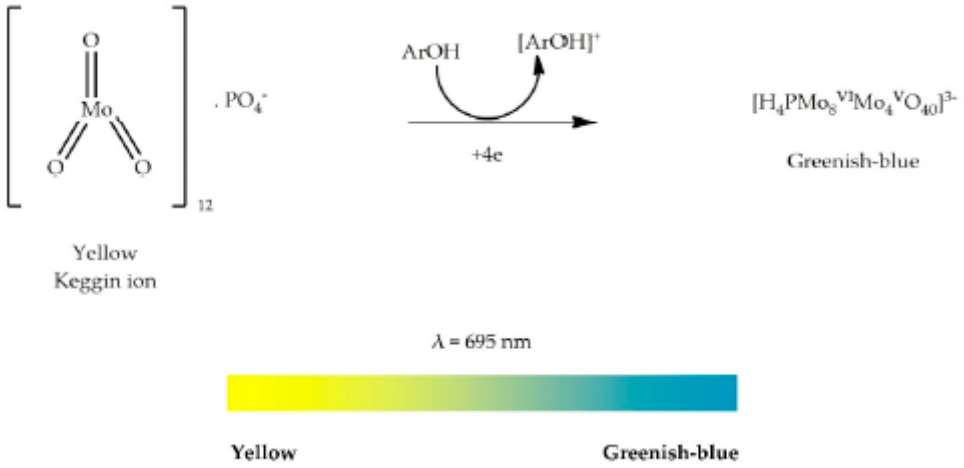

Figure 4: Phosphomolybdenum reaction mechanism.**(Anal. Biochem. 1999)**.

II. Evaluation of the antimicrobial activity:

The evaluation of the antimicrobial activity of the tested essential oil was carried out the research and enumerationof yeasts and molds,the total aerobic mesophilic flora (FATM) andtotal coliforms and fecal coliforms.

II.1. Yeasts and molds in orange essential oil (NF V 08-059):

- **Preparation of EO samples**:

The solutions of the essential oil were prepared with ethanol at 96% and tested at different concentrations.

For this, we prepared a stock solution at a concentration of 0.5g/L. from which the other three solutions were prepared.

- **Procedure:**

The previously melted and cooled Yeast Glucose Chloramphenicol (YGC) agar was distributed in empty Petri dishes. After solidification, 1ml of the essential oil of the orange

was seeded on the surface.1ml of each dilution was spread on the surface. The incubation was done at a temperature of 25°C for 3 to 5 days.

- **Reading:**

Only boxes containing between 15 and 300 colonies are retained for counting.

II.2. the total aerobic mesophilic flora:

The total aerobic mesophilic flora (FMAT), a good indicator of contamination, is enumerated on PCA agar incubated for 48 to 72 hours at 37°C.

- **Procedure:**

From the decimal dilutions, aseptically carry 1ml of the essential oil of the orange and deeply seeded. Then complete with about 15 ml of melted PCA agar (Plate Count Agar) then cooled in the open air. Then make circular and back-and-forth movements in the shape of 8 to allow the inoculums to mix with the agar. Allow solidifying on the bench. The plates will be incubated at 30°C for 72 hours, with a reading every 24 hours. A plate of the medium used was incubated as is, in the same place and under the same temperature conditions; it constitutes the control of the medium **(NF V 08 -059).**

- **Reading:**

Retain the boxes containing a number of colonies between 30 and 300.

The results are expressed in number of germs per "ml" or "g" of product according to the following formula:

$$N = \frac{\Sigma colonies}{v \times d}$$

N: number of germs per ml or g of product.

ΣColonies: sums of the colonies of the interpretable plates.

V: volume of the inoculum (ml)

D: dilution factor considered.

III. Manufacture of the fresh creams incorporated of the essential oil of orange:

The manufacturing process of the elaborated sour creams is illustrated in **Figure05**

The addition of sterile essential oil is made at the level of the microbiological laboratory under sterile conditions. The sampling of the fresh cream is done after the pasteurization step in aseptic conditions. The packaging is done manually in sterile bottles of 50ml bottles, wrapped with aluminum foil .The added essential oil was sterilized by filtration through a sterile filter of 0.45µm

(**MillIpore millex GS)**. The three doses incorporated in the fresh cream are determined from the results of the biological activity tests and preliminary sensory analyses: 0%, X_1%, X_2%, and X_3%.

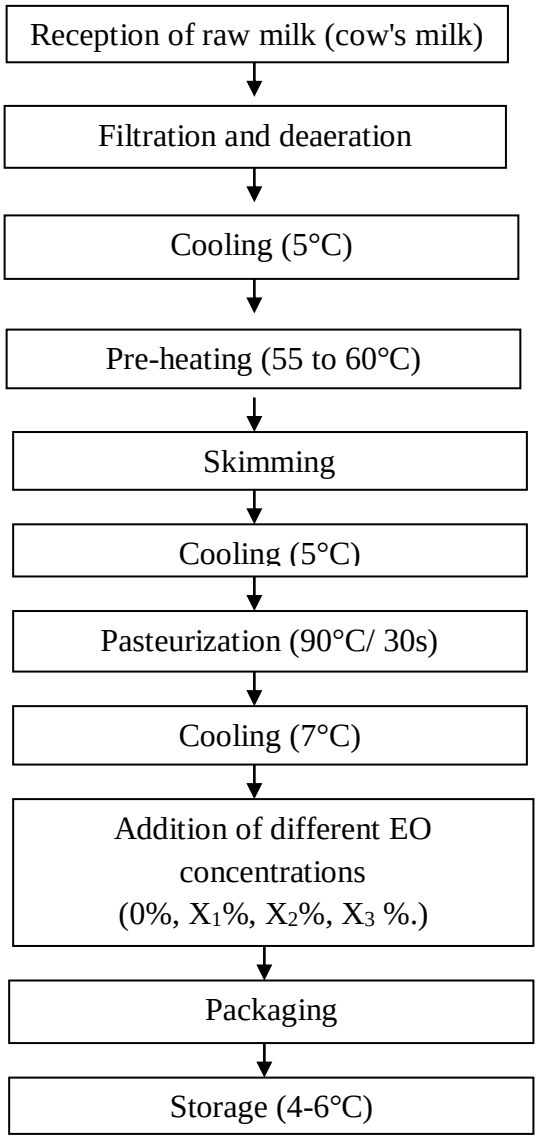

Figure 5: Main steps in the manufacture of sour cream

IV. Physical-chemical analysis:

IV.1. Water and volatile matter content:

The determination of the dry extract was carried out by a desiccator; its principles based on the elimination of total water at a temperature of 125°C for 25 minutes.

A 3g test sample was spread over the entire surface of an aluminum capsule previously tared, then introduced into the desiccators, and the analysis is launched. The dry extract value in (%) is read directly from the digital display after the beep. **(Indicate by the industry to the European standers).**

IV.2.pH:

The measurement of pH was carried out with a pH meter by directly introducing the two probes (pH and temperature) in the samples (milk and sour creams) at a temperature of 20to 25°C.**(AFNOR 1980).**

IV.3.Titratable acidity:

Neutralization of lactic acid in a dairy product by a NaOH solution at N/9 in the presence of a colored indicator (phenolphthalein).

A volume of 10 ml of milk was introduced into a beaker, 2 to 3 drops of phenolphthalein are added, the mixture is homogenized and in the case of sour cream, the 10 ml were diluted into ml of distilled water. From a burette set at 0, the determination of the sample by sodium hydroxide until the pink turn was performed. The pink coloration should persist for at least 10 seconds. The reading of the volume of the used titrating solution is read directly on the burette.

- **Expression of results:**

The acidity of milk and sour creams prepared is expressed in degree Dornic (°D), the degree Dornic degree (°D) corresponds to the number of 1/10 of sodium hydroxide of Dornic N/9 necessary to secure the phenolphthalein **(Guiraud, 2003).**

The acidity is given by the following formula:

$$\textbf{Acidity (°D)} = V \times 10$$

V: the volume in ml of NaOH of the drop read.
1°D= 0,1g of lactic acid.

IV.4. Fat content

The fat content of milk and cream is determined by the method of acid-barometric method described by **AFNOR (1980).** The fat was separated by centrifugation in the butyrometer, after dissolving the proteins by the addition of sulfuric acid. The separation of the fat was favored by the addition of a small quantity of isoamyl alcohol. The fat content was read directly on the butyrometer scale. A volume of 5g of the sample was introduced into a butyrometer (in the case of sour cream. 10ml of distilled water was added), 10ml of sulfuric acid, and 1ml of isoamyl alcohol were added . The butyrometer was closed with a stopper, shaken carefully until lumps disappear, and then introduced into a centrifuge for 15 minutes at speed of 3600 rpm at 65°C.

- **Expression of results:**

The fat content expressed as a percentage was read on the graduated scale according to the following formula:

Fat content (%) = X1 - X 0

X0 = graduation reached by the lower level of the butyrometer;

X1 = graduation reached by the upper level of the butyrometer.

V. Microbiological analysis:

According to the inter-ministerial decree of 27/05/1998, the germs sought and counted in milk and fresh cream are total aerobic mesophilic flora (FTAM) at 30°C; total coliforms; fecal coliforms, yeasts and molds.

V.1.Preparation of stock solutions and decimal dilutions:

For each sample 1 ml of product to be analyzed was introduced into a bottle containing 9 ml of tryptone salt, thus the stock solution corresponding to sour cream, from which decimal dilutions up to were prepared.

V.2. Enumeration of total aerobic mesophilic flora:

Aerobic and facultative aero-anaerobic microorganisms can grow in a non-selective nutrient medium incubated at 30°C for 72 h. They appear as colonies of sizes. A volume of 1ml of stock solution and of each dilution was introduced in empty Petri dishes, on which the nutrient Plate Count Agar (PCA) medium was added. The whole was mixed carefully by rotating in a figure of eight and allowed to solidify. Incubation was performed at 30 °C for 72 hours. After this time, the colonies developed, whatever their size, were counted with a marker.

V.3. Enumeration of total coliforms and fecal coliforms:

The principle consists in counting the characteristic colonies of total coliforms which develop in 24 h at 37°C and fecal coliforms that develop in 24 h at 44°C, on VRBL agar (Violet Red Bile Agar) and then confirm the number of colonies by fermentation of lactose.

In two sets of Petri dishes, transfer 1 ml of sample to be examined and quickly upload 10 to 15 ml of agar medium in super cooling. Then mix carefully by slow rotation in the shape of a figure of eight and let the plates solidify.

The first series was incubated at 37°C for 24/48 hours for the detection of total coliforms

And the second series was incubated at 44°C for 24 hours for the fecal coliforms. After incubation, count the purple-red colonies with a diameter of at least 0.5mm in diameter.

The number of coliform bacteria per gram is given by the following formula:

$$N = c * \frac{1}{d}$$

C: number of colonies;

d: dilution factor

V.4. Enumeration of yeasts and molds:

The isolation of yeasts and molds requires selective media containing antibacterial substances **(Guiraud, 2003).** The melted and cooled YGC (Yeast extract Glucose Chloramphenicol) **ISO, 1988** agar was distributed in empty Petri dishes. After solidification 1ml of each dilution was inoculated on the surface. Incubation was done at a temperature of25°C for 3 to 5 days. The yeasts and molds varied in shape, color, appearance and size **(Sina, 1992).**

VI. Sensory analysis:

The tests commonly used in laboratories for sensory analysis of food products include the study of difference, ranking, intensity rating, and descriptive analyses **(Watts et al., 1991).** The tests applied to our products are the pairwise comparison test, the rank order test, and the hedonic test.

- **Panel:**

The panel consisted of 9 male and female subjects; laboratory technicians and workers in the DANONE Djurdjura factory.

They were shown how the ballots would be filled out, using enlarged ballots projected on a screen. We avoided discussing the food that will be tested, by method and testing protocols used, to reduce confusion and make it easier for the tasters. It is important that they have a clear understanding of the procedures used and how to it is important that they understand the procedures used and how to fill out the forms in order to participate in the tests on the same basis. Tasters should be advised to avoid the use of strong-smelling products such as soaps, lotions, and perfumes before participating in a panel and to avoid eating, drinking, or smoking for at least 30 minutes before testing.

VI.1.Pair-wise comparisons test:

The pairwise comparison test is used to determine the direction of the differences between two samples for the determined property "aroma" and to see if there is a difference between two test samples **(NF V 09-012, 1983).**The principle is the presentation to the subjects of a pair of samples, one of these samples can be the control **(NF V 09-012, 1983).**Six pairs of samples (AB; AC; AD; BC; BD; CD) were prepared. The samples of each pairwere presented to the tasters in cups containing the same quantity of the product and coded byproduct and coded by three-digit ratings. The examiners were asked to fill out the previously prepared form and to rinse their mouths between each pair during the tasting.

- **Analysis of the data:**

The number of responses in the expected direction is totaled, using the table of **Roessler Baker,** and **Amerine** table to analyze the significance of the results.

VI.1. Rank order test:

The purpose of this test is to determine the extent to which the consumer accepts a product. Acceptance of a food product generally indicates the actual consumption of that product .Present a set of samples and ask the tasters to rank these samples coded according to samples coded according to acceptance from least acceptable to most acceptable. A set of four samples (the sour creams prepared) was prepared for each taster, in coded taster, in coded cups. All samples were presented simultaneously to each taster in a random order and tasters in random order, and they were allowed to taste the samples several times.

VI.3. preferably test:

This test was chosen to evaluate the degree of appreciation of the sour cream samples in a general way of sour cream by creating sensory profiles for each product. Ask the tasters to evaluate coded samples of several products by indicating their degree of appreciation on a 9-level scale, in order to create sensory profiles .The four sour creams were presented simultaneously in identical coded cups. The tasters were asked to fill in the forms based on the analysis of the texture, color, smell, taste and aroma of the products.

Results and disscussions

III. Results and discussions:

I. Evaluation of the biological activities of Eos:

I.1.Antioxidant assays:

Several methods have been developed to measure the efficiency of dietary antioxidants. These methods are based on different kinds of defense systems: scavenging reactive oxygen species (ROS), hydroxyl radicals, reduction of lipid peroxyl radicals, inhibition of the lipid peroxidation and chelating of the metal ions **(Achat et al., 2012).** Thus, to evaluate antioxidant effectiveness of EOs, three analytical methods, using different substrates were used. Free radical scavenging properties (DPPH and ABTS), and total antioxidant capacity of orange EOs were presented in **Table 3.**

Table.04: Antioxidant activities of orange essential oil

Activities	Results
DPPH	**I(%)** 26.5±0.87
ABTS	**I(%)** 63.57 ±1.05
Phosphomolybdenum	[C] 14,57±1,02mgAAE/100g

A simple, fast and widely used method. The antioxidant activity of orange EOs is manifested via the change in the color of DPPH- between the oxidized state (purple form) and the reduced state (yellow form), which allows the percentage of inhibition of this radical to be quantified. It is clear that the DPPH antiradical activity of orange EOs is weak and very lower than that of the scavenging effect of ABTS. The antioxidant activity of the tested E.O. is probably related to the majority compounds which are mainly limonene **(Misharina and Samusenko, 2008).** According to **Tang et al. (2001),** β-pinene and limonene have significant antioxidant properties; they minimized the normal rate of a chemical oxidation reaction by scavenging the hydroxyl radical.

The orange EOs inhibition capacity towards ABTS+ radical was 63.57±1.05% that was higher Than the result (50%) obtained by **Torres-Alvarez et al. (2017).** Essential oils are quite complex mixtures constituted by several tens of components and this complexity makes it often difficult to explain the activity pattern. For this reason, many reports on the antioxidant potentials of the essential oils often refer to concepts such assynergism, antagonism and additivity **(Ammar et al., 2012).**

In the total antioxidant capacity, orange EOs was able to reduce the molybdenum (VI) to molybdenum (V) and the formation of a green molybdenum (V) complex with a concentration of 14,57±1,02mgAAE/100g.To the best of our knowledge, there are no data concerning orange EO total antioxidant capacities using phosphomolybdenum assay.

I.2.Microbiological analysis of EOs:

The results of the microbiological analysis of orange essential oil expressed in CFU/ml are represented in **Table 05.**

Table.05: Microbiological analysis of orange EO

Germ	Sample	Orange EO	Standards J.O.A UFC/ml
Yeasts and molds	Pure oil	Absent	-
	Stock solution		
	Control		
FTAMs	Pure oil	Absent	10^5
	Stock solution		
	Control		

The total aerobic mesophilic flora and a good indicator of overall contamination, informs on the hygienic quality of essential oil **(Guinot-thomset al, 1995).** According to **Farris (2009)**, an EO of very good microbiological quality when it contains less than 10^5 germs. This is probably due to the hygienic conditions (extraction equipment and containers).The FTAMs, yeasts and molds of orange essential oil were not detected, wich satisfies the required standard of the Official Journal of the Algerian Republic **(1998)** and **Guiraud (1998).**

II. Elaboration and analysis of enriched sour cream:

II.1.Choice of the incorporation doses of EOs:

On the basis of preliminary tests on sensory analysis of two sour creams, concentrations of Y1%and Y2 %, we have discarded all concentrations that are higher than Y1%, because of the perceived bitterness at this concentration, and lower than Y2 % due to the absence of the perception of the orange EOs aroma. In order to preserve the sensory and conservation criteria of orange EOs and elaborated sour creams, the doses of X1 %; X2 % and X3 % were chosen in this study. This allowed us to elaborate three types of sour cream and control in the dairy industry of "Danone Djurdjura".

III. Physico-chemical analysis:

III.1.Dry extract:

Figure 06 shows the results corresponding to the dry matter content of different analyzed dairy products**.** The histograms revealed that after milk skimming, before pasteurization cream and in the formulated sour creams dry extracts increase considerably (44 % to 48%)compared to the result of raw milk (11 %), which is conform with the data (10-12 %) required by **FAO (2010).**This increase is mainly due to the elimination of milk water after skimming.

The dry extracts were basically the same for the four prepared sour creams, but with a slight decrease compared to cream before pasteurization, that may be explained by the applied heat treatment. Indeed, the pasteurization process reduces the nutritional value of dairy products: the denaturation of part of the proteins, in particular soluble proteins and destruction of water-soluble vitamins (vitamin B; vitamin C) **(Amiot et al., 2002).**

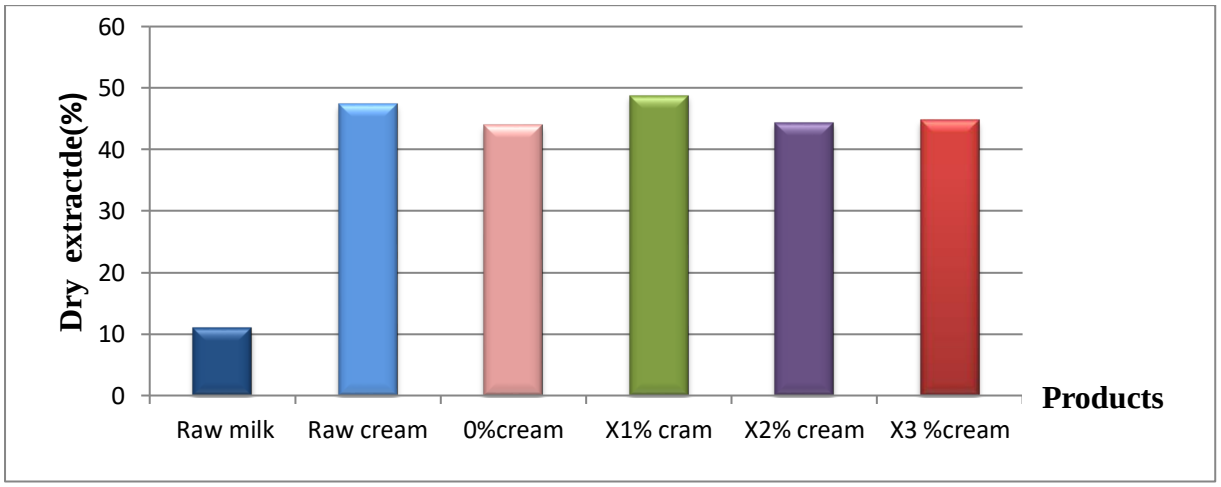

Figure.06: Dry matter content in raw milk, cream and the four prepared sour creams

III.2.Fat content:

The results of fat content in raw milk, cream and the four prepared sour creams, were shown in **figure 07**.The average fat content of the raw milk was 37.5%, this content is slightly lower than the value (39-40 %), recommended by the **OJAR (1993).**

After skimming, the cream obtained was about 39 %, this content decrease after pasteurization to reach 31%, however after the addition of orange essential oil in the sour cream the fat content was gradually rising as a function of the EOs concentrations, until it attains a value of 48%. The elaborated sour creams presented a fat content not conforms with the standards (50-60 %) (**OJAR, 1993)**.

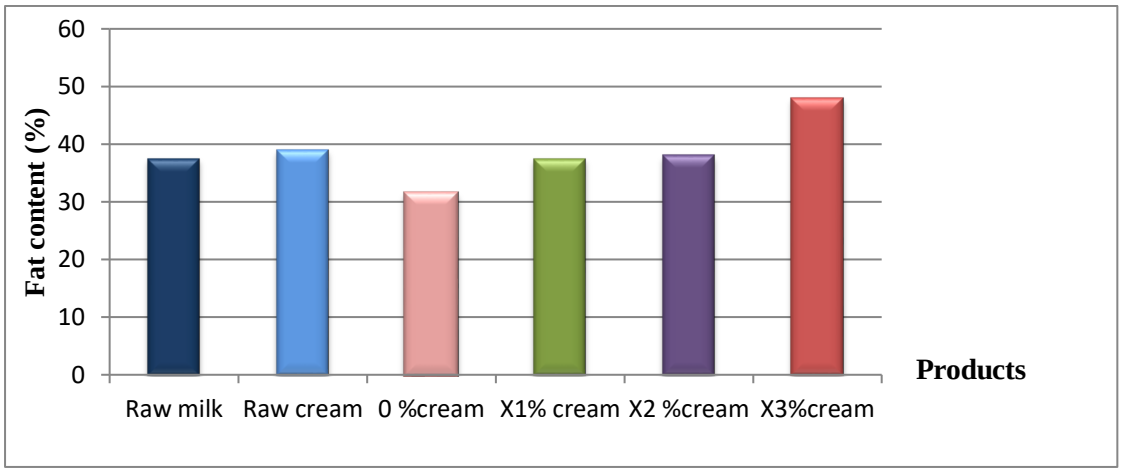

Figure.07: Fat content in raw milk, cream and the four prepared sour creams

III.3.pH:

The evolution of the pH during the production of sour creams and after the packaging **Figure 08** revealed three main phases, in the first one stationary phase(raw milk pH = 6,68) where we did not perceive any pH variation after skimming(raw cream pH = 6.67 and final product).However, in the second one a decline pH phase was observed after pasteurization (final product)and which continue to decrease in the third one phases after 2 and 4 days of opening, that depend on the incorporated EOs rate. This decline in pH is probably due to the unwinding and a dissociation of the protein chains under the effect of the heat treatment while releasing H+ ions. This drop in pH continues even after opening the package but at a higher rate following a hypothetical fermentation due to microbial contamination after sour cream uses.

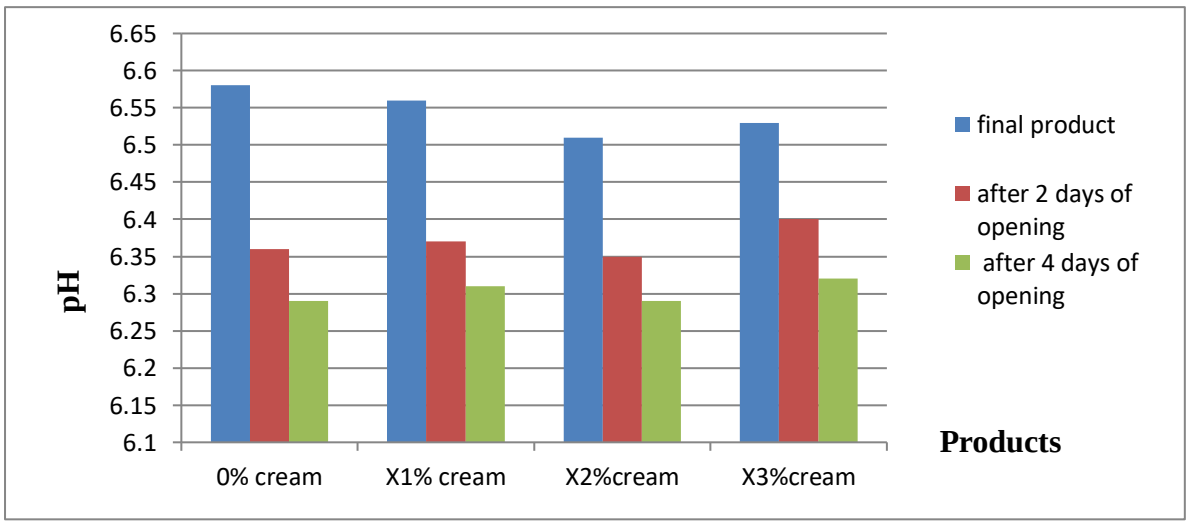

Figure.08: Evolution of pH during the production of sour creams and after the packaging

III.4.Titratable acidity:

The monitoring of titratable acidity during the production of sour creams and after the packaging was given in **figure 09.**

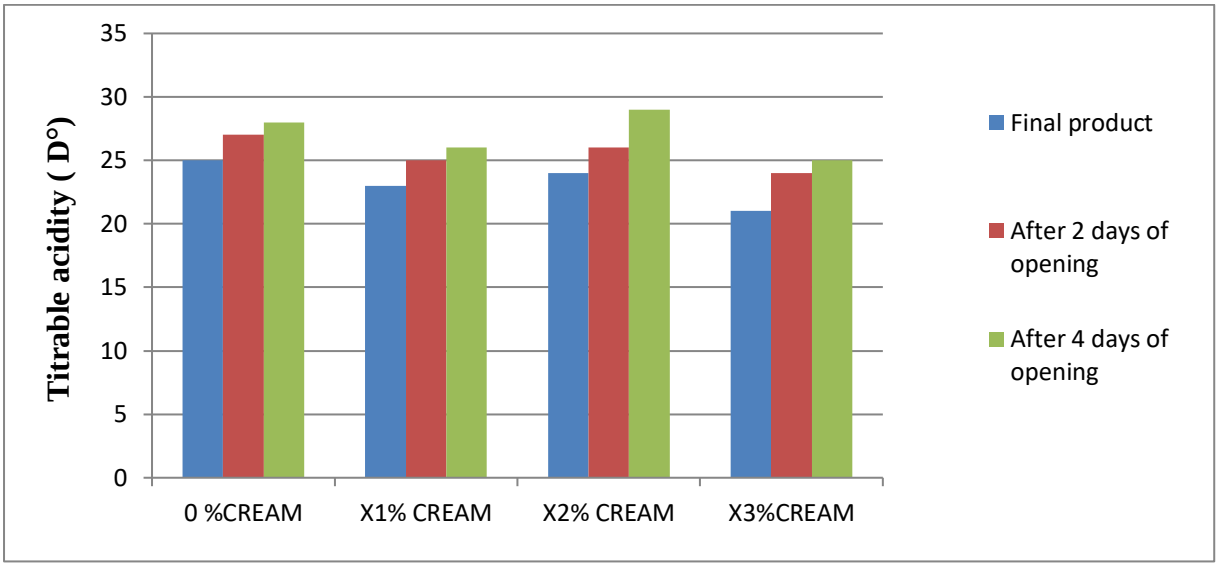

Figure.09: Evolution of titrable acidity during the production of sour creams and after the packaging

The results revealed that skimming slightly increase the titratable acidity (raw milk TA = 18°D and raw cream TA = 21°D), and the same tendency was observed after cream pasteurization and after addition orange EOs and which continue to increase, gradually, after 2 and 4 days of opening where sour cream (X3%) presents a value of TA 29°D.

These results indicate the influence of the presence of the essential oil of Citrus sinensis on the cream. It can help to increase its titratable acidity with the possibility of preventing the growth of lactic bacteria that ferment lactose into lactic acid, during storage.

According to figures 08 and 09 of the variation of titrable acidity and pH during the preparation of sour creams and after the opening of the package, we notice that these two parameters are reversely balanced: the higher titratable acidity, the lower pH.

IV. Microbiological analysis:

The results of the microbiological analysis of raw milk and cream before pasteurization were presented in table 06. These data showed:

- That the total coliforms and fecal coliforms of two studied products were not detected, thus conforms with norms(**OJAR, n°35/1998)**;
- The absence of molds in cream before pasteurization (conform with norms);

- That raw milk and cream before pasteurization contain an important microbial flora (FATM, yeasts) and molds for molds for only raw milk, that far outnumber the norms, indicating the unsanitary quality of used the raw material.

Table.06: Hygienic quality of raw milk and cream before pasteurization

Germs (CFU/ml)	Raw Milk	Cream before Pasteurization	Norms CFU /ml
FATM	6.10^6	5.10^3	10-300
Total coliforms	ND	ND	<150
Fecal coliforms	ND	ND	<150
Yeasts	6.10^4	8.10^1	30-300
Molds	2.10^4	ND	30-300

The microbiological analysis of elaborated sour creams, after opening during 3 days, was conducted and the results were given in table 07.

Table.07: Evolution of the hygienic quality of sour creams, after the opening during 3 days

		FATM CFU/ml	Total coliforms CFU/ml	Fecal coliforms CFU/ml	Yeast CFU/ml	Molds CFU/ml
0%	**D1**	300	0	0	0	0
	D2	450.10^4	0	0	8	0
	D3	150.10^4	0	0	1100	0
X1 %	**D1**	230	0	0	0	0
	D2	7.10^5	0	0	10	0
	D3	2.10^5	0	0	2400	0
X2 %	**D1**	300	0	0	0	0
	D2	420.10^5	0	4.10^3	25	0
	D3	130.10^5	0	0	180	0
X3 %	**D1**	200	0	0	0	0
	D2	150	0	0	40	0
	D3	60	0	0	120	0

Molds, yeast and coliforms are the primary contaminants in dairy products **(Amakoromoet al. 2012)**, were not detected in sour creams samples (0 %, X1 %, X2 % and X3) at day 1 (D1). However, FTAM was recorded,but witha rate that was conform to the standards. This illustrates the adequate heating treatment of milk under strict aseptic conditions, during processing and manufacturing of the different enriched sour creams samples.

The results of the second and the third day (for all prepared sour cream), showed that total coliforms, fecal coliforms, yeast and molds were conforms with the value required by

OJARn°35/ (1998), except for sour cream enriched with X2 % orange EOs, about fecal coliforms at day 2, and yeasts at the day 3 for sour creams (0 % and X1 %) which did not correspond to the standards value (**OJAR, n°35/1998)**.

Nevertheless, an important growth of FATM was found in the second day after opening, in all sour cream samples,indicating that these products are unsafe for consumptionafter 48 hours. Suddenly, a drastic decrease was observed in mesophilic flora at the day 3, for all manufactured sour creams that depend on the incorporated EOs concentration.

The follow-up of the count of FATM in the sour cream at X3 % EOs allowed us, clearly to notice the reduction in the number of FATM compared to the other concentrations. From these results, the addition of orange essential oils to sour cream preparations, did not exhibit an antibacterial effect at day 2 on FATM, but this activity appeared on the third day after opening.

As an outcome, the shelf life of the sour cream after opening (after 48 hours) failed to be preserved by the orange EOs in it at various concentrations. The product began to degrade as soon as the packaging was opened on the second day. On the other hand, this observation might be explained by the low EO concentrations used, or by the fact that the meal matrix in this instance consists of two lipidic and one aqueous phase. Essential oils diluted in food's lipidic phase will have less of an impact on the aqueous phase's microorganisms **(Mejlholm and Dalgaard, 2002)**. As a result, the EO's interaction with macromolecules like lipids or proteins safeguards the microorganism from the EO's action **(Tassouet al., 1995).** A chemical interaction involving proteins and the functional groups of EO decreases the number of active molecules that are available **(Malecky, 2007).**

V. Sensory analysis:

Four samples of sour creams A. B. C and D (A = 0%, B = X1 %, C =X2 % and D = X3 %), were sensory evaluated and scores were recorded.

V.1.Pairwise comparison test:

The results of the pairwise comparison test are shown in Table 08. The critical value for the difference test was 9.Panel members were able to detect the significant difference (number of correct responses = 10 > 9) between the fourth samples pairs. This indicates that the incorporation of EO at different concentrations resulted in significantly different products. The aromatization threshold was analyzed and the results show that only the pairs (A-B), (A-C), (A-D) present a significant difference in flavoring (number of detections of difference in flavoring = 10 > 9) indicating that each addition of the EO induces a perception of aromatization compared to the control even at low concentration (X1 %). As for the three other preparations, the

detection of the difference in aromatization was not significant and the degree of aromatization was perceived indifferently. No significant preference was revealed between the control and the other three preparations flavored with orange EO; even between the different flavorings concentrations no significant difference was revealed.

Table.08: Results of the pairwise comparison test

Pair	Correct answer; difference	Flavored product	Preferred product
A-B	10	10	5A-5B
A-C	10	10	5A-5C
A-D	10	10	5A-5D
B-C	10	7	5B-5C
C-D	10	5	5C-5D
B-D	10	8	5B-5D
Critical value	9	9	9
Conclusion	There is a significant difference for all six pairs	There is a significant difference for the first 3 pairs	There is no significant difference forthe six pairs

V.2. Ranking test:

We asked the tasters to rank the four sour cream preparations (A, B, C and D) in terms of acceptability without giving a tie, giving each sample a different rating even though they seemed comparable. The sample with the most acceptable taste was given a rating of 1, the next most acceptable was given a rating of 2, the least acceptable was given a rating of 3, and the last one was given a rating of 4. The ratings given to each sample by the 10 tasters are summarized in **Table 09.**

Table.09: Results of the rank order test of the sour cream samples

Taster	A	B	C	D
1	4	1	2	3
2	2	1	4	3
3	2	1	4	3
4	3	1	2	4
5	4	1	2	3
6	4	1	2	3
7	3	2	1	4
8	2	1	3	4
9	3	1	2	4
10	2	1	3	4
Ranking Totals	29	11	25	35
Critical value = 15				

The critical value, calculated for 10 tasters and 4 samples, is 15 according to the table in **Appendix 04**.The X3 % EO concentration resulted in a significantly downgraded product compared to the control. The X1% concentration was the most acceptable compared to the other concentrations and also to the control, with a difference of 18 tasters. The tasters ranked the four samples in order of preference according to the intensity of taste, giving first place to (B), followed by the control A in second place and the concentration C in third place. Finally, the most flavored processed sour cream (D) was ranked fourth.

V.3. preferably test:

The figures gather the sensory profiles of the four samples with different indicators (**Figure 10**):

- ✓ The texture
- ✓ The smell
- ✓ The taste
- ✓ The aroma

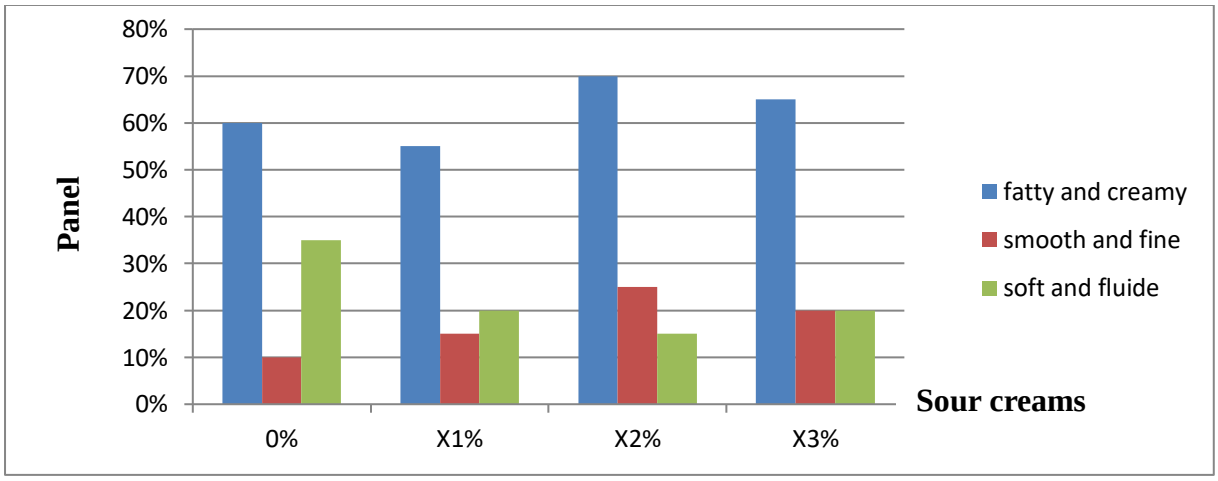

Figure.10: The results of the appreciation of the elaborated sour cream according to the texture

Figure shows the sensory profiles of the four cream samples, we note that the tasting panel members perceive that the sensory characteristics describing the orange aroma and smell were more intense in all sour creams containing the orange EOs. In contrast, no remarkable differences were noted for the other attributes.

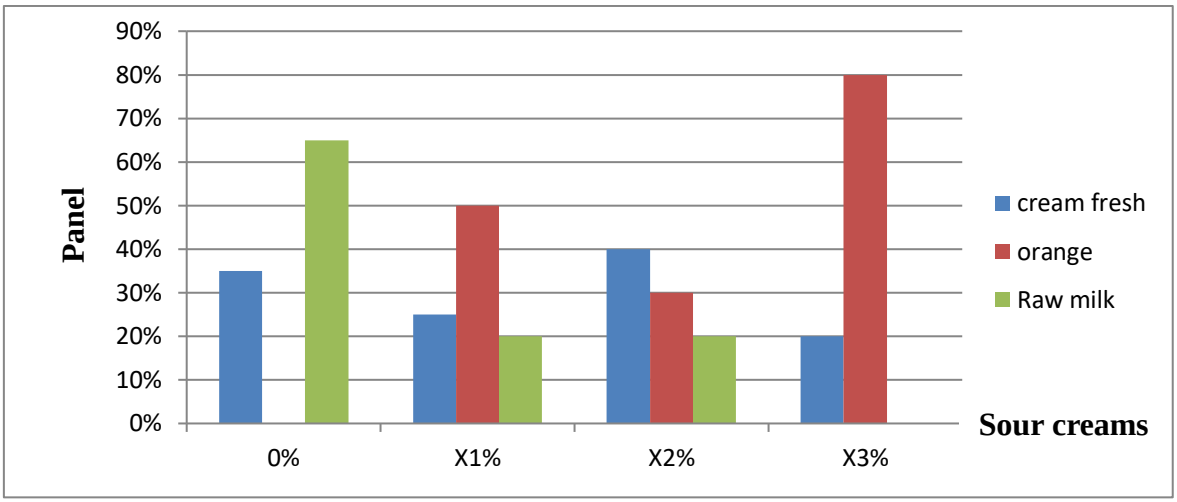

Figure.11: The results of the smell and the aroma of the elaborated sour cream samples

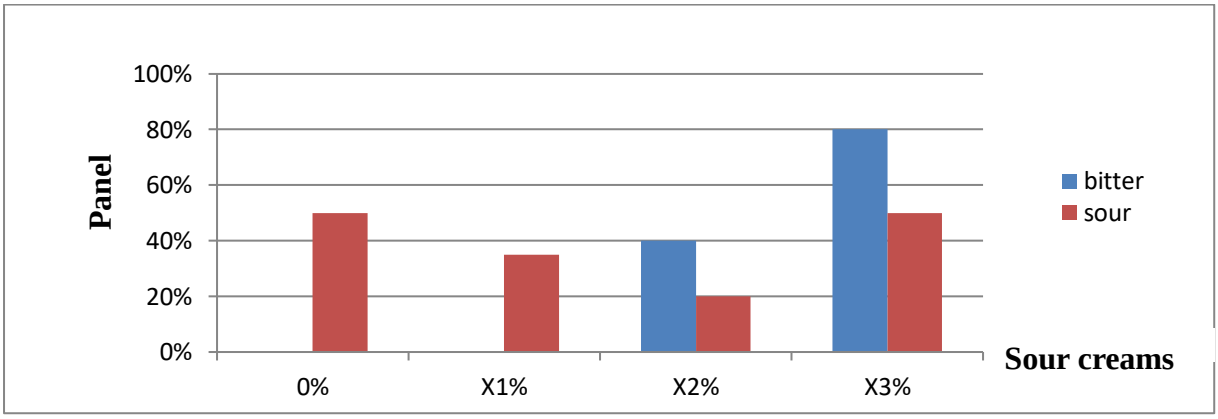

Figure.12: The drop results of the elaborated sour cream samples

These results (**Figure 11 and 12**) allow us to conclude that the incorporation rates of the EO in the sour creams modify the aroma and the smell, without touching the characteristics of the texture and the color. The degree of acceptability of these sour creams with the orange EOs to the tasters is maintained, since there was no significant difference during the classification of the four studied sour creams. The concentration of X3 % EO gave a product significantly downgraded compared to the control.

Conclusion

Conclusion:

We recall that the objectives of this study ought to enrich sour cream with orange essential oil on the one hand, and to expand the various types of existing sour cream on the other, by evaluating antibacterial, antioxidant, and aromatic effects. In the course of this study, we were able to identify the following findings:

The estimation of the antioxidant activity of orange essential oil by reduction methods of DPPH°, ABTS and, showed that this essential oil does not really have an important antioxidant power, for this reason, many reports on the antioxidant potentials of the essential oils often refer to concepts such as synergism, antagonism and additivity. Therefore, its use in sour cream may not constitute a possible means of preventing lipid oxidation.

The microbiology of the orange essential oil of showed a hygienic quality; free of FTAM and yeasts and molds which allows us to incorporate it in the processed sour cream at different concentrations (X_1%, X_2% and X_3%).

The various tests concerning the elaboration of the sour creams enriched with the orange essential oil were carried out. The characteristics are in accordance with the standards. The results of chemical and microbiological analysis of the sour creams, after opening the packaging, revealed that the presence of essential oil at low concentrations does not limit the microbial alteration.

The outcome of the sensory analysis demonstrates that the enrichment of the sour cream with the essential oil, for concentrations of X_1% and X_2%, does not lead to any difference from the point of view of aromatization, and give products which are classified indifferently with the control. Only the incorporation rate of X_3% essential oil significantly downgraded the product compared to the control in third place, which led to changes in odor and aroma while keeping the characteristics of texture, color and taste the same.

From all these results, we can conclude that orange EO seems to be more suitable as an antioxidant and aromatic agent in sour cream.

Although this work has studied the biological characteristics of orange EO and explored the possibility of its use as a preservative and flavoring agent in sour cream, many questions would deserve to be addressed, so it is desirable:

Conclusion

- To evaluate the antimicrobial activity of orange essential oil, using different bacterial strains.
- Evaluation of the antioxidant activity of orange essential oil by the β-carotene bleaching assay, and manufactured sour creams
- Oxidative stability of sour creams.
- To study the effect of the essential oil of on the lactic ferments of the sour cream;
- Isolate lactic acid bacteria from sour cream and study their behavior towards the essential oil of citrus sinensis.
- Determination of the chemical indexes
- To identify the constituents of the essential oil of citrus sinensis

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The appendixes

Appendix01: Orange Essential oil.

Appendix 02: Presentation of the Dairy Danone Djurdjura Algeria

Danone Djurdjura Algeria was born in 2001, it is the fruit of a beautiful meeting between a world leader of fresh dairy products DANONE and the Algerian dairy Djurdjura which was created by the BATOUCHE Family in 1983 and which had a sustained growth for 20 years. Their mission is to bring health through food to as many people as possible. Danone is one of the world's leading food companies and relies on four businesses: Fresh Dairy Products and Plant-based Products, Baby Nutrition, Waters and Medical Nutrition.

Danone Djurdjura Algeria, a subsidiary of the Danone Group, currently employs 800 Danoners divided between a factory in Akbou (Béjaïa), a headquarters in Algiers and a distribution center in Tessala El Merdja.

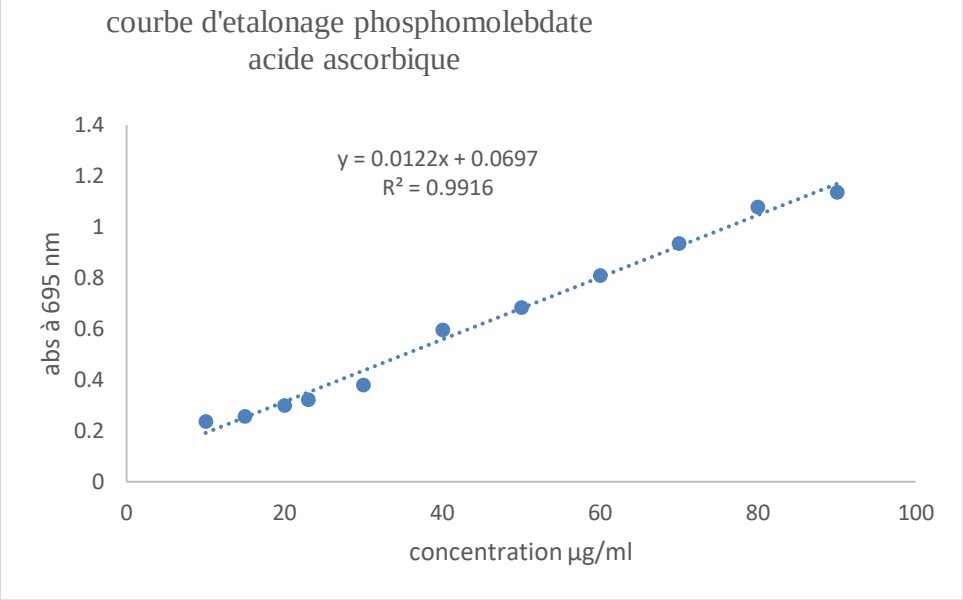
At Danone Djurdjura Algeria, their concern is to offer safe, healthy and quality products while contributing positively to the Algerian society, in line with the group's vision "One Planet, One Health". And this thanks to the support of their partner breeders who supply us with quality raw milk.

The company is very interested in the development of the milk sector in Algeria by accompanying their partner breeders via the program "H'lib Dzair" in order to train them and make them more productive.

Through their Infant Nutrition division, they support parents and more particularly mothers by providing them with food and nutritional advice for the growth of their children. They are committed to promoting exclusive breastfeeding following the recommendations of the World Health Organization

DANONE
ONE PLANET . ONE HEALTH

Appendix 03: Phosphomolepdate calibration chart ascorbic acid.



Appendix 04: The petri dishes of orange essential oil samples for microbiological enumeration and detection of yeasts and molds and enumeration of FTAM in orange EO.

Appendix 05: filtre 0, 45 (MILLIPORE MILLEX GS).

Appendix 06: pairwise comparison test bulletin.

Date :
Nom :
But de l'essai :
Critère en essai

N° Dégustateur :

Paires en essai	**Y a-t-il une différence ?**	**Quel est l'échantillon le plus aromatisé ?**	**Quel est l'échantillon préféré ?**
Code n° /code n°			
…. **….**	Oui Non	…….	…….
…. **….**	Oui Non	…….	…….
…. **….**	Oui Non	…….	…….
…. **….**	Oui Non	…….	…….
…. **….**	Oui Non	…….	…….
…. **….**	Oui Non	…….	…….

Appendix 07: Roessler, Baker and Amerine table for pairwise comparisons tests.

DEGUSTATEUR	DIFFERENCE			PREFERENCE		
	5%	1%	0, 1%	5%	1%	0,1%
7	7	7	-	7	-	-
8	7	8	-	8	8	-
9	8	9	-	8	9	-
10	9	10	10	9	10	-
11	9	10	11	10	11	11
12	10	11	2	10	11	12
13	10	12	13	11	12	13
14	11	12	13	12	13	14
15	12	13	14	12	13	14
16	12	14	15	13	14	15
17	13	14	16	13	15	16
18	13	15	16	14	15	17
19	14	15	17	15	16	17
20	15	16	18	15	17	18
21	15	17	18	16	17	19
22	16	17	19	17	18	19
23	16	18	20	17	19	20
24	17	19	20	18	19	21
25	18	19	21	18	20	21
30	20	22	24	21	23	25
35	23	25	27	24	26	28
40	26	28	31	27	29	31
45	29	31	34	30	32	34
50	32	34	37	33	35	37
60	37	40	43	39	41	44
70	43	46	49	44	47	50
80	80	51	55	50	52	56
90	54	57	61	55	58	61
100	59	63	66	61	64	67

Appendix 08: Rank test bulletin.

FICHE DE TEST DE CLASSEMENT

N° Dégustateur :

NOM :…….

-Veuillez classer les quatre échantillons par ordre de

DATE :……

Code	Classement

Appendix09: Differences in critical absolute rank sums for comparaisons of all treatments at a 1% sifnificance level.

	nombre d'échantillon									
Dégustateurs	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
3	6	8	11	13	15	18	20	23	25	28
4	7	10	13	15	18	21	24	27	30	33
5	8	11	14	17	21	24	27	30	34	37
6	9	12	15	19	22	26	30	34	37	42
7	10	13	17	20	24	28	32	36	40	44
8	10	14	18	22	26	30	34	39	43	47
9	10	15	19	23	27	32	36	41	46	50
10	11	15	20	24	29	34	38	43	48	53
11	11	16	21	26	30	35	40	45	51	56
12	12	17	22	27	32	37	42	48	53	58
13	12	18	23	28	33	39	44	50	55	61
14	13	18	24	29	34	40	46	52	57	63
15	13	19	24	30	36	42	47	53	59	66
16	14	19	25	31	37	42	49	55	61	67
17	14	20	26	32	38	44	50	56	63	69
18	15	20	26	32	39	45	51	58	65	71
19	15	21	27	33	40	46	53	60	66	73
20	15	21	28	34	41	47	54	61	68	75
21	16	22	28	35	42	49	56	63	70	77
22	16	22	29	36	43	50	57	64	71	79
23	16	23	30	37	44	51	58	65	73	80
24	17	23	30	37	45	52	59	67	74	82
25	17	24	31	38	46	53	61	68	76	84
26	17	24	32	39	46	54	62	70	77	85
27	18	25	32	40	47	55	63	71	79	87
28	18	25	33	40	48	56	64	72	80	89
29	18	26	33	41	49	57	65	73	82	90
30	19	26	34	42	50	58	66	75	83	92
31	19	27	34	42	51	59	67	76	85	93
32	19	27	35	43	51	60	68	77	86	95
33	20	27	36	44	52	61	70	78	87	96
34	20	28	36	44	53	62	71	79	89	98
35	20	28	37	45	54	63	72	81	90	99
36	20	29	37	46	55	63	73	82	91	100
37	21	29	38	46	55	64	74	83	92	102
38	21	29	38	47	56	65	75	84	94	103
39	21	30	39	48	57	66	76	85	95	105
40	21	30	39	48	57	67	76	86	96	106
41	22	31	40	49	58	68	77	87	97	107
42	22	31	40	49	59	69	78	88	98	109
43	22	31	41	50	60	69	79	89	99	110
44	22	32	41	51	60	70	80	90	101	111
45	23	32	41	51	61	71	81	91	102	112
46	23	32	42	52	62	72	82	92	103	114
47	23	33	42	52	62	72	83	93	104	115
48	23	33	43	53	63	73	84	94	105	116
49	24	33	43	53	64	74	85	95	106	117
50	24	34	44	54	64	75	85	96	107	118
55	25	35	46	56	67	78	90	101	112	124
60	26	37	48	59	70	82	94	105	117	130
65	27	38	50	61	73	85	97	110	122	135
70	28	40	52	64	76	88	101	114	127	140
75	29	41	53	66	79	91	105	118	131	145
80	30	42	55	68	81	94	108	122	136	150
85	31	44	57	70	84	97	111	125	140	154
90	32	45	58	72	86	100	114	129	144	159
95	33	46	60	74	88	103	118	133	148	163
100	34	47	61	76	91	105	121	136	151	167

Appendix10: Raport card for the hedonic test with a rating scale from 1to 9**.**

FICHE DE TEST
HEDONIQUE

N° Dégustateur :

NOM :……..
PRENOM :……..
DATE :……
Veuillez examiner et goûter chaque échantillon de fromage, et donnez une note de1 à 9 selon l'intensité du caractère.

Echantillon

		A	B	C	D
Texture	Liquide				
	Grasse				
	Crémeux				
	Onctueuse				
	Fluide				
	Fine				
	Souple				
	Nappent				
Couleur	Jaune				
Odeur	Levuré				
	Fromage				
	Herbe vert				
	Fruit sec				
	Orange				
	De rance				
Goût	Acide				
	Salé				
	Sucré				
	Amère				
Arôme	Fromage				
	Orange				
	Noisette				
	Lait cru				
	Fruit sec				

Abstract:

The aim of our study is to highlighting the conservative and aromatization role of the essential oil of Citrus Sinensis on sour cream. Three concentrations of the EO of Citrus Sinensis equal to X_1%, X_2% and X_3% have been incorporated to the sour cream that has made from cow's milk (raw milk).

This oil has a quiet insignificant antioxidant activity (inhibition of almost 26.5 % of free radical) and results the inhibition capacity of the ABTS + radical by the studied EO, after six minutes of incubation is 63.57 % at a concentration of 0,40mg/ml and a14, 57mgAAE/100g±1, 02 of The total antioxidant capacity (TAC) The results were expressed as mg ascorbic acid equivalent per g of dry matter.

The effect of the EO has been evaluated by following the evolution of the physicochemical and microbiological parameters during 14 days of storage, +2 UBD and +4 UBD.

The various tests on the formulation of the added essential oil of Citrus Sinensis creams were tested by developing three sour creams with concentrations of X_1%, X_2% X_3% Characteristics are conforms to standard. The result of physicochemical and microbiological analyzes prepared sour cream after opening the package, show that the presence of the essential oil at low concentrations does not limit microbial spoilage.

At the sensory analysis, the incorporation of the orange essential oil into the sour cream at concentrations of X_1% and X_3%, involves no significant difference in terms of flavoring, and make products which are classified in same order than the control. Only the rate of incorporation of the essential oil of X_3% significantly downgraded the product compared to the control in the third row, which has led to changes in smell and flavor while the texture, color and taste are unchanged.

Keywords: Citrus Sinensis, orange essential oil, sour cream, antimicrobial, antioxidant, Sensory analyses.

Résumé :

L'objectif de notre étude est de mettre en évidence le rôle conservateur et aromatisant de l'huile essentielle de Citrus Sinensis sur la crème aigre. Trois concentrations de l'HE de Citrus Sinensis correspondant à X1%, X2% et X3% ont été incorporées dans de la crème aigre fabriquée à partir de lait de vache (lait cru).

Cette huile a une activité antioxydante insignifiante (inhibition de près de 26,5 % du radical libre) et les résultats la capacité d'inhibition du radical ABTS + par l'HE étudiée, après six minutes d'incubation est de 63,57 % à une concentration de 0,40mg/ml et de 14,57mgAAE/100g±1,02 de la capacité antioxydante totale (TAC) Les résultats ont été exprimés en mg d'équivalent acide ascorbique par g de matière sèche.

L'effet de l'HE a été estimé en suivant l'évolution des paramètres physico-chimiques et microbiologiques pendant 14 jours de stockage, DLC+2 et DLC +4.

Les différents tests sur la formulation des crèmes additionnées d'huile essentielle de Citrus Sinensis ont été testés en élaborant trois crèmes aigres avec des concentrations de X1%, X2% X3% Les caractéristiques sont conformes à la norme. Le résultat des analyses physico-chimiques et microbiologiques de la crème aigre préparée après ouverture de l'emballage, montre que la présence de l'huile essentielle à de faibles concentrations ne limite pas l'altération microbienne.

En ce qui concerne l'analyse sensorielle, l'incorporation de l'huile essentielle d'orange dans la crème aigre à des concentrations de X1% et X3%, n'entraîne aucune différence significative en termes d'arôme, et donne des produits qui sont classés dans le même ordre que le témoin. Seul le taux d'incorporation de l'huile essentielle de X3% dégrade significativement le produit par rapport au témoin dans le troisième rang, ce qui a modifier l'odeur et la saveur alors que la texture, la couleur et le goût restent inchangés.

Mots-clés : Citrus Sinensis, huile essentielle d'orange, crème aigre, antimicrobien, antioxydant, analyses sensorielles.