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The evaluation of the supplementation effect of natural extracts on the sensory quality of raw milk

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#### List of abbreviations

% percentage

ADSA American Dairy Science Association

BIS Bureau of Indian Standards

C Centigrade

CFU colony-forming unit

cm Centimeter

EO Essential oil

g Gram

GC Gaz chromatography

H+ Ion hydrogen

HTST high-temperature short-time

Kg Kilogram

L Litter

m Meter

MFG milk fat globules

mg milligram

mL Milliliter

PCA Principal components analysis

pH hydrometric potential

s second

S Sulfur

sH Soxhlet-Henkel degrees of titratable acidity

TPM Texture profiling method

UHT ultra-high-temperature

## Introduction

#### Introduction

Better production, better nutrition, and a better environment, these three points were the driving forces behind the United Nations Summit on Food Systems, which took place in September 2021 under the auspices of the Food and Agriculture Organization of the United Nations (FAO). The organization's purpose is no longer confined to eradicating hunger and promoting food security but also to guarantee healthier and more natural food (Figeczky et al., 2021).

Following this new path, applying natural food additives has become a sustainable development trend; compared with their synthetic analogs, which are known for their harmful effects on human health and the environment, natural food additives have garnered considerable attention owing to their advantages such as green safety, health, and environmental protection. Therefore, the development and utilization of natural food additives is becoming the most active field and the future direction for the development of the food industry (Zang et al., 2022).

Of all their different resources, natural plant-based additives have gained popularity in recent years due to the wealth of the green kingdom, which has a diverse range of active compounds whose combination opens up a world of possibilities. Rosmarinus and Origanum have been the subject of many studies about the effect of adding both their aqueous extracts and essential oils to different aliments (Petter, 2004)

Nieto et al. (2018) tested the effect of using rosemary as a food additive; their study warrants the introduction of rosemary extracts or essential oils, with high phenolic compound contents, into the food industry. Gad and Sayd (2015) have studied the potential uses of rosemary as a natural antioxidant in dairy products; according to their study, polyphenolic compounds in rosemary are significant contributing factors to the limited shelf life as well as possibly health-protecting compounds. However, this natural antioxidant can negatively affect the sensory quality of dairy products.

As for oregano, Rychen and al. (2017) have used its essential oil as a food additive for different animals, and they were able to identify the optimum concentrations that guarantee its safety and efficacy. Boroski et al. (2012) tested the use of oregano essential oil as an antioxidant in dairy products for human nutrition; they found out that omega-3 fatty acid

in the EO effectively inhibits the oxidation during storage. However, their study reveals that the use of oregano negatively impacts sensory properties, so before adopting this supplementation, consumer acceptance has to be evaluated.

In this context, our present study aims to determine the sensory effect of supplementing raw milk with "Rosmarinus officinalis L" and "Origanum vulgare L" aqueous extracts. Our strategy consists of evaluating the global sensory quality in order to compare the effects of the two plant extracts, then dissecting these global scores to point out the variables responsible for the differences in order to relate them with the chemical composition of the two plants. We will be using different concentrations for each sample which will allow us to determine the effect of the concentration on the sensory quality.



# Chapter 1 Sensory analysis

#### 1. Generalities on sensory analysis

Quality is an old and recurring concern in the food industry and remains at the forefront of consumers' interests. The term "quality" for food products refers to several factors, including nutritional, sanitary, and organoleptic. As a result, the food industry focuses on these three critical aspects of quality (Potter and Hotchkiss, 1995).

The interest in organoleptic qualities is recent; it only began in the early 1900s in different food and beverage industries with the use of professional tasters and consultants (Clark et al., 2009).

Sensory evaluation was first used officially between the 1940s and the mid-1950s by the U.S. Army Quartermaster Food and Container Institute to provide acceptable food to the military services. Right after this experiment, the food sector quickly jumped on board, realizing that sensory evaluation could contribute pertinent, valuable information related to marketing consequences and simultaneously provide directly actionable information (Stone and Sidel, 2004).

Sensory science as we know it has evolved into a set of quantitative procedures that enhance the efficiency and accuracy of food product development, quality control, market research, and marketing. This development has been realized by combining sensory evaluation with analytical procedures and incorporating tests as a formalized, structured, and codified methodology (Clark et al., 2009).

#### 2. Definition

Sensory evaluation has been defined as a scientific method used to evoke, measure, analyze, and interpret responses to products as perceived through the senses of sight, smell, touch, taste, and hearing. This definition has been accepted and endorsed by sensory evaluation committees within various professional organizations such as the Institute of Food Technologists and the American Society for Testing and Materials (Lawless and Heymann, 2010).

#### 3. The objective of sensory evaluation

The basic purpose of sensory evaluation is to determine food's organoleptic qualities and the way they affect human subject actions by stimulating different sensorial cephalic receptors (Sztrygler et al.,1990).

Based on the nature of the chosen sensory test, these effects may have a wide range of consequences and may constitute helpful information for different disciplines in interaction with sensory evaluation such as chemistry, food science, marketing, etc. (Lawless and Heymann, 2010).

Hence, sensory evaluation may have different objectives (Branger et al., 2007; Darke, 2007; Deneulin and Pfister, 2013):

- Describing products in order to establish a sensorial profile;
- Understanding consumer preferences;
- Studying the impact of manufacturing conditions;
- Tracking the effects of storage and determining an optimal date limit of use;
- Product maintenance;
- Product improvement/optimization;
- Development of new products;
- Assessment of market potential;
- Product category review;
- Support for advertising claims.

#### 4. Principles of sensorial methodology

To properly situate the sensory evaluation, it is necessary first to understand the phenomenon of sensory perception by clarifying certain basics:

#### 4.1. Sensory attributes

Sensory attributes are the parameters used in the sensory analysis of a food item. By their order of perceiving, we can find:

#### 4.1.1. Appearance

Appearance is a very important attribute; it's often used as a principal characteristic to base a marketing decision. Sensory analysts must pay special attention to every detail of the test sample appearance. Appearance allows making judgments concerning: color, size and shape, surface texture, and Carbonation (Lawless and Heymann, 2010).

#### 4.1.2. Odor/aroma/fragrance

The odor of a product is detected when its volatiles enters the nasal passage to be perceived by the olfactory system. We talk about the odor when the volatiles are sniffed through the nose, aroma for food product odor, and fragrance for the odor of perfume or cosmetic (Meilgaard et al., 2016).

#### 4.1.3. Consistency and texture

The texture is perceived by sensors in the mouth, the skin, and the body's muscles (Meilgaard et al., 2016). According to the physical condition of the tested product, we distinguish:

- Viscosity (for homogeneous Newtonian liquids);
- Consistency (for non-Newtonian or heterogeneous liquids and semisolids);
- Texture (for solids or semisolids).

#### 4.1.4. Flavor

Flavor has been defined as the sum of perceptions resulting from stimulation of the sense ends grouped at the entrance of the alimentary and respiratory tracts. However, for purposes of practical sensory analysis, the term is restricted to the impressions perceived via the chemical senses from a product in the mouth (Caul, 1957). Following this path, flavor includes (Meilgaard et al., 2016):

- The aromatics, i.e., olfactory perceptions caused by volatile substances released from a product in the mouth via the posterior nares;
- The tastes, i.e., gustatory perceptions (salty, sweet, sour, bitter) caused by soluble substances in the mouth;
- The chemical feeling factors that stimulate nerve ends in the soft membranes of the buccal and nasal cavities (astringency, spice heat, cooling, bite, metallic flavor, umami taste).

#### 4.1.5. Noise

The noise produced during mastication of foods or handling fabrics or paper products is a minor, but not negligible, sensory attribute. Measuring the pitch, loudness, and persistence of sounds produced by foods or fabrics is common. The pitch and loudness of the sound contribute to the overall sensory impression. Differences in the pitch of some rupturing foods (crispy, crunchy, brittle) provide sensory input that can be used in assessing freshness/staleness. Oscilloscopic measurements permitted sharp differentiation between products described as crispy and those described as crunchy. Kinesthetically, these differences correspond to measurable differences in hardness, denseness, and the force of rupture of a product. A crackly or crisp sound on handling can cause a subject to expect stiffness in a fabric. The duration or persistence of sound from a product often suggests other properties, e.g., strength (crisp fabric), freshness (crisp apples, potato chips), toughness (squeaky clams), or thickness (plopping liquid) (Vickers and Bourne, 1976).

#### 4.2. The human senses:

The sensory evaluation uses the human senses as instruments of perception allowing for the collection of different sensory information. Each sense organ is specialized for perceiving certain types of data, which, together, are used to describe a complete profile of the product to test (Lawless and Heymann, 2010).

The following graphic (figure 1) summarizes the human senses and the perception process of different sensory attributes.

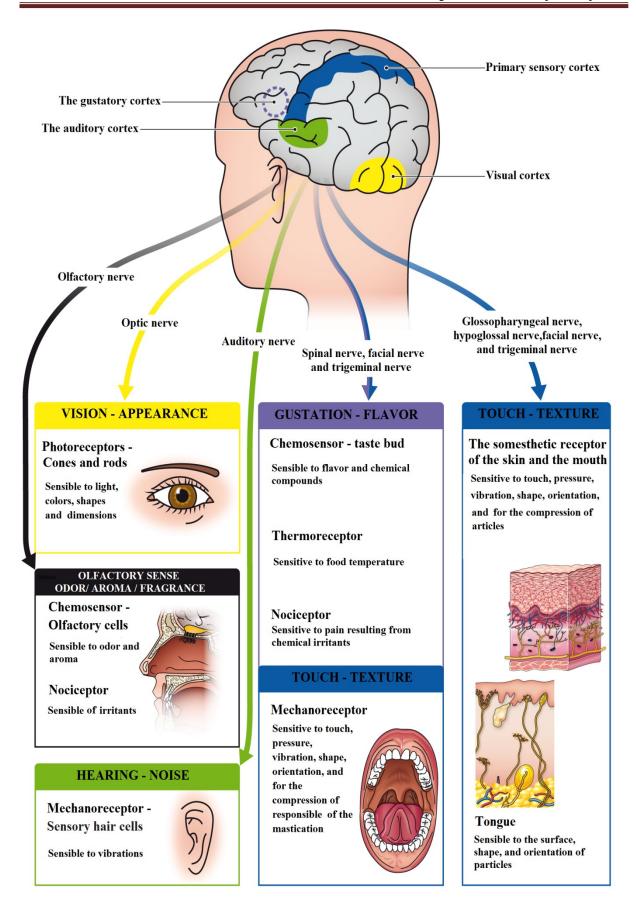


Figure 1: summary of the sensitive organs' activity (S.Léger, 2013)

#### 4.2.1. The sense of vision

Light enters the eye's lens and being focused on the retina, where the rods and cones convert it into neural impulses that travel to the brain via the optic nerve. Sensory analysts must pay attention to certain aspects (Meilgaard et al., 2016):

- Subjects are influenced by adjoining or background color and the relative sizes of areas of contrasting color; blotchy appearance affects perception.
- The gloss and texture of a surface also affect the perception of color.
- Color vision differs among subjects; degrees of color blindness exist,
   exceptional color sensitivity also exists.

#### 4.2.2. The sense of touch

The sense of touch can be divided into (Geldard, 1972):

#### Somesthesis (tactile sense, skin feel)

The somesthetic sensations are responsible for touch, feeling pressure, heat, cold, itching, and tickling. The nerve ends in the skin surface; epidermis, dermis, and subcutaneous tissue are responsible for perceiving these kinds of sensations.

#### **Kinesthesis (deep pressure sense or proprioception)**

Kinesthetic perceptions corresponding to the mechanical movement of muscles (heaviness, hardness, stickiness, etc.) result from stress exerted by muscles, jaw muscles, or tongue and the sensation of the resulting strain (compression, shear, rupture) within the sample being handled or masticated. Kinesthesis is felt through nerve fibers in muscles, tendons, and joints.

#### 4.2.3. The olfactory sense

The olfactory epithelium in the roof of the nasal cavity is covered by millions of tiny, hair-like cilia responsible for sensing airborne odorants. Optimal contact is obtained by moderate Inspiration (sniffing) for 1–2 s (Laing 1983). At the end of 2 s, the receptors have adapted to the new stimulus, and one must allow 5–20 s or longer for them to de-adapt before a new sniff can produce a full-strength sensation (Meilgaard et al., 2016).

Sensory testers must pay attention to certain aspects (Harper 1972):

- The odorants can fill the test's location, reducing the subject's ability to detect a particular odorant or differences among similar odorants.
- Specific anosmia, the inability to detect specific odors, is not uncommon. For this reason, potential panelists should be screened using odors similar to those to be eventually tested.

#### 4.2.4. The Chemical/Trigeminal Sense

Often confused with olfactory and/or gustatory sensations, chemical irritants such as ammonia, ginger, horseradish, onion, chili peppers, menthol, etc. stimulate the trigeminal nerve ends, causing perceptions of burn, heat, cold, pungency, etc. in the mucosa of the eyes, nose, and mouth. For most compounds, the trigeminal response requires a concentration of the irritant that is orders of magnitude higher than one that stimulates the olfactory or gustatory receptors (Meilgaard et al., 2016).

#### 4.2.5. The Sense of Gustation/Taste

Gustation is a chemical sense that involves the detection of stimuli dissolved in water, oil, or saliva by the taste buds located on the tongue's surface as well as in the mucosa of the palate and areas of the throat (Drewnowski, 2001).

The prudent taster should take small sips and keep each sip in the mouth for only a couple of seconds, then wait (depending on the perceived strength) for 15–60 s before tasting again. The first and second sips are the most sensitive.

The gustatory sensors are bathed in a complex solution, the saliva, and they are fed and maintained by a second solution, the blood. Hence, humans can only taste differences in the concentration of many substances, not absolute concentrations, and their sensitivity to levels (e.g., of salt) that are lower than those in the saliva is low and ill-defined (Clark et al., 2009).

#### 4.2.6. The Sense of Hearing

Vibrations in the local medium cause the eardrum to vibrate. The vibrations are transmitted via the small bones in the middle ear to create hydraulic motion in the inner ear fluid, the cochlea, which is a spiral canal covered in hair cells that, when agitated, sends neural impulses to the brain (Clark et al., 2009).

Recently, scientists are more interested in the concepts of intensity and pitch of sound waves. They are more aware of the importance of controlling sources of noises and vibrations (Civille and Setsam, 2003).

#### 5. Sensory evaluation test methods:

The primary concern of sensory evaluation is to ensure that the test method is appropriate to answer the questions about the tested product (Lawless and Heymann, 2010). For this reason, tests are usually classified according to their primary purpose and most proper use. Three types of sensory testing are commonly used, each with a different goal and each with participants selected using different criteria:

#### **5.1. Difference Testing**

Simple difference testing procedures also called discrimination tests, aim to tell whether any perceptible difference exists between two types of products. Their analyses are usually based on the statistics of frequencies and portions (counting right and wrong answers). From the test results, we infer differences based on the proportions of persons who can choose a test product correctly from a set of similar or control products. There are three types of discrimination tests: The triangle test, the duo–trio test, and the paired comparison test (Lawless and Heymann, 2010).

#### **5.1.1.** The triangle test:

The triangle test was used in the Carlsberg breweries and the Seagrams distilleries in the 1940s (Helm and Trolle, 1946; Peryam and Swartz, 1950). In this test, two products are from the same batch while a third product is different. Judges would be asked to pick the odd sample from among the three. Ability to discriminate differences would be inferred from consistent correct choices above the level expected by chance. This test can serve as a means to screen judges with sufficient discrimination abilities for different evaluation methods (Meilgaard et al., 2016).

#### 5.1.2. The duo-trio procedure

Another multiple-choice difference test was developed at about the same time in distilleries for quality control purposes. A reference sample is given, and then two test samples. One of the test samples matched the reference while the other is from a different

product. The participant would try to match the correct sample to the reference, with a probability of one-half. As in the triangle test, a proportion of correct choices above that expected by chance is considered evidence for a perceivable difference between products (Lawless, 2013).

#### **5.1.3.** The paired comparison

In this third popular difference test participants are asked to choose which of two products is stronger or more intense in a given attribute. Partly because the panelist's attention is directed to a specific attribute, this test is very sensitive to differences (Lawless, 2013).

#### **5.2.** Descriptive analyses

The second major class of sensory test methods is those that quantify the perceived intensities of the sensory characteristics of a product. These procedures are known as descriptive analyses (Lawless and Heymann, 2010).

#### **5.2.1.** The Flavor Profile method

The first method with a panel of trained judges, it was originally developed by scientists at Arthur D. Little, an international management consulting firm, in the late 1940s and has been used extensively to describe the flavor of foods (Caul, 1957).

In essence, the flavor profile describes flavor in terms of 5 major components: character notes or attributes, intensities of those attributes, the order of appearance of the attributes, aftertaste, and amplitude. The original scale for the flavor profile was 5 points: not present, threshold, slight, moderate, and strong. Over time the scale has been adapted to more points to accommodate more intensity differentiation (Keane, 1992).

#### 5.2.2. The texture profile method

The Texture Profile Method was developed by scientists working for General Foods in the 1960s and was based on the flavor profile method. Initially, Szczesniak (1963) developed a texture classification system that proposed to bridge the gap between expert and consumer texture terminology, classifying perceived texture into three groups, "mechanical", "geometric", and "other" characteristics; the classic TPM was then based on this classification. This technique aims to allow the description of texture from the first bite through complete mastication and also accounts for the temporal aspect of attributes. Attributes in TPM are rated on the Szczesniak scale, which covers the range of sensations in

foods, and scale points are anchored with specific food products. The original TPM used an expanded 13-point scale; however, TPM panels have recently been trained using category, line, and magnitude estimation scales (Meilgaard et al., 1991).

#### 5.2.3. Quantitative descriptive analysis

Quantitative Descriptive Analysis was developed during the 1970s by Tragon Corporation under partial collaboration with the Department of Food Science at the University of California, Davis. Initial intentions for this method were to deal with statistical treatment on data obtained by Flavor Profile and related descriptive methods (Stone et al., 1974).

Quantitative Descriptive Analysis allows the evaluation of multiple products by making relative judgments with high precision since humans are good at judging relative sensory differences but poor at evaluating absolute ones. This philosophy has made this methodology distinctly different from those descriptive methods which try to finalize the absolute difference among products (Stone et al., 2012).

#### 5.2.4. The quantitative flavor profiling technique

Quantitative flavor profiling was developed by Givaudan-Roure, Switzerland as a modified version of the quantitative descriptive analysis. This technique concentrates on the description of flavor only. In addition, it uses a common standardized technical flavor language which makes it highly suitable for cross-cultural or cross-laboratory projects, developed by a panel of 6–8 people who are typically flavorists and not directly involved in the project (Murray et al.,2001).

Quantitative flavor profiling has been used for the flavor profiling of dairy products, particularly cheeses, yogurt, and sweetened milk (Stampanoni, 1994).

#### 5.2.5. The spectrum method

Gail Vance Civille developed the spectrum descriptive analysis method during the 1970s, this method incorporates the rigor of the training and structure of the flavor and texture profile methods and then adds a complete spectrum to be treated with a more refined scale [over 150 points of discrimination] using more sophisticated statistical methods to the descriptive data. (Meilgaard et al., 2016)

#### 5.2.6. Generic descriptive analysis

Also called hybrid descriptive analysis, it allows the most suitable methods to be used and combined according to the needs of the project and the specific research requirements (Murray et al., 2001).

#### **5.2.7.** Free-choice profiling

Free-choice profiling is a quick and inexpensive method in which consumers are asked to both identify attributes in the sample and rate the liking and/or intensity of those attributes. They should be provided with adequate instruction on how to perform this test and possibly given product categories to consider (aroma, appearance, flavor, texture, etc.). Each consumer will have different attributes, indicating which are most important. Though consumers should be recruited as normal (product usage, age/gender specifications), researchers may be able to separate consumers into groups, better identifying which characteristics are most important in that segment (González et al.,2001).

#### **5.3.** Affective testing

Affective tests can be classified into two main categories based on the primary task of the test:

#### **5.3.1. Preference Tests**

A preference test is indicated if a project is specifically designed to pit one product directly against another in situations such as product improvement or parity with the competition. The preference test forces a choice of one item over another or others. It does not indicate whether any of the products are liked or disliked. Therefore, the researcher must have prior knowledge of the "affective status" of the current product or competitive product it is testing against (Meilgaard et al., 2016).

#### **5.3.2.** Acceptance Tests

The acceptance test is used when there is a need to determine the "affective status" of a product, i.e., how well consumers like it. The product is compared to a well-liked company product or with a competitive product, and a hedonic scale is used to indicate degrees of appreciation from unacceptable to acceptable (Clark et al., 2009).

As a conclusion of this chapter, we can say that sensory analysis is an essential step in developing new products; it allows the evaluation and control of the sensory qualities of food. As a typical example, dairy products were among the very first products to be the subject of such analysis because of their wide use and fragile properties. Our next chapter will be dedicated then to dairy products, their properties, and the variants of their organoleptic quality.

### Chapter 2

## The organoleptic aspect of milk

#### 1. Generalities on milk

Milk is a nutrient-rich liquid food produced by the mammary glands of mammals. It is the primary source of nutrition for young mammals (including breastfed human infants) before they can digest solid food (Aishwarya and Duza, 2017).

Human milk consumption was defined in 1909 by the International Congress of Food by the following formula: "milk is the product of the total, full and uninterrupted milking of a dairy female in good health, also nourished and not overworked. It must be collected properly and not contain colostrums" (Guetouache et al., 2014).

Milk has a crucial role in healthy human nutrition and development, especially in childhood. It is a nutrient-dense food; it supplies energy and high-quality protein with a range of essential micronutrients in an easily absorbed form (Wu et al., 2014).

#### 2. Raw milk compared with pasteurized milk

The European Commission issued article 1992/46 defined raw milk as milk that is not heated to more than 40 degrees Centigrade during processing. In addition, the EU regulation stipulates hygienic standards from the animal and the farm to the market. It also imposes standards for microbial levels in the raw product that farmers have the responsibility to monitor subject to agreement by the buyers (Bingen and Busch, 2006).

Because of its high water content, neutral pH, and biochemical composition, raw milk is a good medium for microbial development. As a result, raw milk may contain a variety of microorganisms with different classifications, morphologies, and physiologies. Bacteria in raw milk can cause spoiling or be pathogenic, depending on whether they are mesophilic, psychrophilic, or thermophilic (Özer and Akdemir-Evrendilek, 2015).

In order to reduce the number of pathogenic microorganisms to safe limits for consumers' health, various heat treatments are used. The most common include low and high pasteurization (Lewis, 2003). However, heat treatment affects the molecular structure of milk proteins at the interfaces of oil-in-water emulsions and in aqueous media (Raikos, 2010)

#### 3. Milk organoleptic properties

To ensure quality and safety, raw milk should be examined and controlled along with its production process. The quality of raw milk is related to its composition, physicochemical (density, pH, acidity, etc.), and sensory properties (color, odor, taste, flavor, texture, and mouthfeel) (Bloksma et al., 2008).

#### 3.1. Flavor

Over the past five decades, numerous researches have been dedicated to analyzing dairy products' flavors and how they affect consumers' acceptance. Sensory evaluations have proven that consumer acceptance of milk as a beverage is influenced by its flavor more than any other attribute. Good quality fresh milk has a bland yet characteristic flavor. It has a slightly salty and sweet taste due to salts and lactose. The American Dairy Science Association lists the following flavor criticisms: acid, astringent, barny, bitter, cooked, cowy (acetone), feed, fermented/fruity, flat, foreign, garlic/ anion (weedy), lacks freshness (stale), malty, oxidized (metal-induced), oxidized (light-induced), rancid (lipolysis), salty and unclean (psychrotrophic), as well as the corresponding sensory characteristic (Nursten, 1997).

#### 3.2. Appearance

In contrast to flavor, consumers tend to paid less attention to the appearance characteristics, particularly the color of milk and dried milk, this might be because the color of milk is generally taken for granted and does not become a quality issue until it is altered from its normal or expected state. Fluid milk varies in color from opaque white to yellowish-white or even to a blueish tinted white. The observed color of milk is attributed to the light reflectance properties of the milk components such as fat globules, colloidal substances (e.g., proteins), carotene, and riboflavin (Solah et al., 2007).

#### 3.3. Texture

Milk, skim milk, cheese whey, and whey permeate are dilute solutions and are usually considered Newtonian fluids with a pleasant mouthfeel (kincella, 1984)

The viscosity of milk and dairy products, in general, is related to their composition, the casein micelles of milk contribute more to the viscosity than any other constituent. Hydration of protein can also cause an increase in viscosity, and obviously water; a higher amount of water decreases concentration and viscosity. All these factors are used by the dairy

products industry to control the desired level of viscosity by varying fat content level, heat-treating, homogenizing, cooling, and packaging (Robinson, 2005).

#### **3.4. Odor**

Typical milk has a very little distinct odor. However, because of its blandness, milk is an effective vehicle for off-odors; the lability of some of its components, and odorous compounds are readily generated from milk by hydrolysis, oxidation, and enzymic and microbial activity (Nursten,1997).

Moio and al (1993) have used Charm Analysis (Combined hedonic response measurement) to identify 14 active odor volatiles in freshly secreted bovine, ovine, caprine, and water buffalo milk. Of the active odor volatiles, 8 were present in all kinds of milk. Ethyl butanoate and hexanoate (fruity aroma) were the major contributors among the neutral odorants.

#### 4. Factors affecting milk organoleptic properties

The sensory properties of milk are affected by several factors; we can site chemical composition, physical properties, microbiological properties, nutrition, and milk processing.

#### 4.1. Chemical composition

#### **4.1.1.** Lipids

Triglyceride is the major lipid component of cow's milk (98% of milk fat). The other 2% of milk lipids consist of diglycerides, monoglycerides, cholesterol, phospholipids, free fatty acids, cerebrosides, and gangliosides (Robinson, 2005).

Milk fat plays a critical role in the sensory perception of fluid milk. It is preferred by all consumer segments at varying levels and is considered to be a contributor to creaminess, which is positively correlated with product liking (McCarthy et al., 2017).

Visual, texture, and flavor attributes of milk are all influenced by milk fat. Descriptive sensory analysis of fluid milk of varying fat percentages demonstrated that opacity, thickness, mouthcoating, viscosity, milk fat flavor, and yellow color increased with fat content (Frøst, 2001).

#### 4.1.2. Proteins

Regular bovine milk contains about 3.5% protein, based on the classification suggested by the American Dairy Science Association (ADSA) in 1984, these proteins can categorized into caseins, whey proteins, proteins involved in the formation of the membrane that surrounds milk fat globules (MFGs), and enzymes. Casein molecules are in colloidal dispersion in the aqueous phase of the micelles in milk, known as casein micelles, generally retained by ions, mainly calcium and phosphorus. The whey proteins are the milk proteins that remain in the serum after the precipitation of caseins at pH = 4.6 °C and 20°C (Varzakas and Tzia, 2016).

Protein contents contribute to the sensory properties of fluid milk; Strecker aldehydes can be produced via amino acids and, in turn, may lead to the corresponding alcohols. Cysteine and methionine, free or combined in peptides or proteins, are the source of sulfur compounds, such as hydrogen sulfide, methanethiol, and dimethyl disulfide. The casein micelles of milk contribute more to the viscosity of milk than any other milk constituents.. Milk enzymes also have an impact on the sensory properties, their specific action causes the coagulation of milk which alters its texture, and abusive handling of raw milk may result in a rancid flavor from the action of the naturally occurring lipase enzyme, which breaks down butterfat to free fatty acids (i.e., butyric acid is perceived as "rancid") (McSweeney et al., 2016; Robinson, 2005).

#### 4.1.3. Salts

Chlorides, phosphates, citrates, sulfates, potassium, calcium, magnesium, and sodium bicarbonates make up the majority of milk salts. Some milk salts (such as chlorides, sulfates, sodium, and potassium) are soluble and practically fully present in milk whey as ions. Others, like calcium and phosphate, are significantly less soluble; they exist partly associated with the casein micelles. The proportion of calcium phosphate in the dissolved and colloidal forms has a big impact on milk's characteristics (Walstra and Jenness, 1984).

Trace minerals in milk impart a salty background taste to fluid milk that is not directly noticeable but can be more clearly perceived in milk or whey permeates. Sodium and potassium, the largest contributors to salty taste, are found in milk at 391 to 644 and 1.212 to 1,681 mg/kg, respectively (Muehlhoff and al.,2013).

#### 4.1.4. Lactose

Lactose is the main carbohydrate in milk, with a concentration of 4.8 % in cow's milk. This level does not make milk unduly sweet because lactose is less sweet than an equimolar mixture of its components, galactose, and glucose. As a result, lactose hydrolysis produces sweeter milk (Schulz and Rizvi, 2021).

Lactose contributes to the colligative properties of milk (osmotic pressure, freezing point depression, boiling point elevation), which gives a naturally smooth texture for milk. Compared with many other sugars, lactose is relatively less soluble in water; its solubility at 25°C is only 17.8 g/ 100 g solution (Robinson, 2005). Lactose is also responsible for certain flavors; maltol and 2-acetylfuran, used primarily as a flavor enhancer, are derived from lactose without a bacterial intervention (Nursten,1997).

#### 4.1.5. Other components

Milk also contains many vitamins (e.g., vitamins A and C), enzymes (e.g., lactoperoxidase and acid phosphatase), and somatic cells. Some minor constituents may perform an important sensory function, and others may be accidental contaminants causing an alteration of the natural properties (Robinson, 2005).

#### 4.2. The microbiology of raw milk

Milk is typically sterile upon secretion within the secretory glands and is presumably first contaminated with bacteria within the teat canal. Milk handling in the farm results in further contamination and growth of spoilage organisms. Sources of microbiological contamination on the farm include equipment used for automated milking, milk handling lines, and refrigerated bulk milk storage tanks (Özer and Akdemir-Evrendilek, 2015).

Raw milk microflora can be grouped as indigenous or contaminants and also as spoilage or pathogenic microorganisms.

#### 4.2.1. Indigenous microflora

Normally the udder of a healthy animal is habited by bacteria that belong to genera Streptococcus, Staphylococcus and Micrococcus which account for >50% of overall raw milk flora, followed by *Corynebacterium*, *Escherichia coli* and others. Microbial counts of aseptically drawn milk is < 100 CFU mL<sup>-1</sup>, but in practice, they usually range from > 1000 CFU mL<sup>-1</sup> to 20.000 CFU mL<sup>-1</sup> (Özer and Akdemir-Evrendilek, 2015).

#### 4.2.2. Contaminant microorganisms

After secretion, the initial microbial load of raw milk changes because microorganisms from different sources enter the milk. The contaminant microorganisms, which belong to different genera, are distributed as follows: *Lactobacillus, Corynebacterium, Microbacterium, Pseudomonas, Escherichia, Alcaligenes, Acinetobacter, Bacillus, Clostridium*, yeasts, and molds at levels of <10%, Lactococcus and Streptococcus at varying levels from 0 to 50% and Micrococcus and Staphylococcus at levels varying from 30 to 99 % (Chambers, 2005).

In general, according to Özer and Akdemir-Evrendilek (2015), both indigenous and contaminant microflora of raw milk are classified into two categories:

- The spoilage microorganisms responsible for the spoilage or the deterioration of milk properties.
- The pathogenic microorganisms responsible for provoking animal or human diseases.

Whether the contamination occurs before or after pasteurization, microbes can significantly impact milk flavor (Santos et al., 2003).

Microorganisms in raw milk can have an impact on the sensory quality of milk and may even cause premature milk spoilage; Lipases and proteases released from some bacteria can cause free fatty acid flavor and bitter taste, mastitis can develop unpleasant sensory defects such as rancidity and bitterness, *Streptococcus lactis var. maltigenes* produces malty flavors, *Pseudomonas perolens* produces pyrazine chemicals that create musty potato odors (Özer and Akdemir-Evrendilek, 2015).

Pasteurized milk can also be affected by microorganisms, psychrotrophic gramnegative rod bacteria are responsible for the majority of post-pasteurization contamination of fluid milk characterized by a fruity (pineapple or strawberry-like) off-flavor as well as lower levels of sour, rancid, and soapy flavors. Spores, typically from gram-positive rods such as Paenibacillus and Bacillus species, are also of concern for pasteurized milk (Fromm and Boor, 2004). Spores can survive HTST pasteurization and cause sensory spoilage within 25 to 30 day even when total bacteria counts are below 20.000 CFU/mL (Barbano et al., 2006).

### 4.3. Physical characteristics

Milk is an extremely complex biological fluid with scores of nutrients contained in a fluid with three physical phases: an emulsion, a colloidal dispersion, and a solution. The physical properties of milk have a big impact on its sensory properties. Thus, they are being used for assessing and monitoring the quality of products, such as yogurts, cream, butter, and cheese (Chandan et al., 2009).

## **4.3.1. pH** value

The Soxhlet-Henkel degrees (SH), which represent the titratable acidity value of fresh cow milk is between 6.4 and 7.0. This is due to acidic salts and free organic acids (mostly citric acid); casein, which reacts with NaOH as an acid to maintain this balance. The formation of lactic acid by specific bacteria, particularly lactic acid bacteria, increases the SH value and causes milk coagulation. In raw milk, SH levels of less than 5.0 indicate mastitis, feeding problems, or negative microbial impacts, resulting in diminished lab coagulation or milk flavor abnormalities. When the SH value is between 8.0 and 9.0, the milk has a perceptible sour flavor, and when the SH value is greater than 10, protein coagulation occurs during the heating process. SH levels of 10-12 will not always result in heat precipitation, as breeding activity can result in a greater value. Because the SH analysis cannot exactly determine the dissociated fraction of the acid, the pH value must be used to calculate the acidity (Aiello et al 2019; Clark et al, 2009).

### 4.3.2. Viscosity

The viscosity is the main characteristic of milk's texture. It is around 2.2 to 2.5 mPa.s at 20 °C, which depends on the individual cow's metabolism and state of nutrition. Along the production process, milk is standardized to the desired fat content, heat-treated, homogenized, cooled, and packaged. All of these factors can significantly affect the viscosity of the final product and change its texture properties (Chandan et al., 2009).

### 4.3.3. The surface properties

Surface properties are another critical characteristic of milk's texture. The surface tension of milk approximates 70% of that of water (72 dynes/cm). The surface tension of cow's whole milk ranges from 50 to 52 dynes/cm. and for skim milk, 55–60 dynes/cm at 20 °C. This property is used to follow the changes in surface-active components during milk

processing, to follow the release of fatty acids during lipolysis, and to measure the foaming tendency of milk (Chandan et al., 2009).

# 4.3.4. Thermal properties

Heat transfer plays an important role in many dairy processing operations, and in most cases, it is desirable to maximize the rate of heat transfer. At a temperature range of 5–40 °C, the coefficient of thermal expansion of milk with standard composition is around 0.335 cm<sup>3</sup>Kg<sup>-1</sup>C<sup>-1</sup>. Milk has a thermal conductivity of 193 J M<sup>-1</sup> s<sup>-1</sup> K<sup>-1</sup> at 37 °C and 223 J M<sup>-1</sup> s<sup>-1</sup> K<sup>-1</sup> at 80 °C. With an increase in fat or total solids, thermal conductivity drops dramatically. (McCarthy, 2002 ).

The effect of heat on milk could alter its appearance properties by making its color darker due to the Maillard reaction. Heat affects the flavor as well, over 80°C; milk protein's peptides chains unfold, thus denaturing irreversibly. This deformation enables the thiol groups to interact with other molecules forming S–S bonds hydrogen sulfide, methanethiol, dimethylsulphide, and carboxylsulphide. These molecules are responsible for the "cooked" off-flavor developed during UHT and high-temperature pasteurization (Varzakas and Tzia, 2016).

Heat treatment can also alter texture properties, depending on the pH of the environment and the proximity of molecules available; whey proteins can be incorporated into the MFG membrane and casein micelles (Penna et al., 2006).

### 4.4. Animal factors

### **4.4.1.** Genetic

The potential fat content of milk, protein, and lactose levels from an individual cow are determined genetically; milk composition varies considerably among dairy cattle breeds: Jersey and Guernsey breeds give milk of higher fat and protein content than Shorthorns and Friesians, Zebu cows can give milk containing up to 7% fat. Heredity also determines the potential milk production of the animal. Thus, selective breeding can be used to upgrade milk quality.. (Angulo et al., 2006).

# 4.4.2. Stage of lactation

The stage of lactation affects milk protein and fat percentages very similarly. In colostrum, the highest amount of protein and fat in milk is found just after freshening. Levels

drop to their lowest point between 25 and 50 days after calving and peak at 250 days as milk production decreases (Auldist et al., 1996).

### 4.4.3. Age

Age tends to cause both milk fat and protein to decline as the animal becomes older. Milk fat falls about 0.2% each year from the first to fifth lactation. Protein decreases by 0.02 to 0.05 % each lactation as animals age (Garamu, 2019).

### 4.5. Environmental Factors

### 4.5.1. **Season**

Milk composition includes milk fat, milk protein, dry matter, and non-fat milk solids, the decreases and changes of which lead to the reduced quality of milk. A previous study reported that, in dairy cows, high temperatures caused the variations in milk composition (Peana et al., 2007)

Milk fat and protein percentages are highest during the fall and winter and lowest during the spring and summer. This variation is related to changes in the types of feed available and climatic conditions. Lush spring pastures low in fiber depress milk fat. Hot weather and high humidity decrease dry matter intake and increase feed sorting, resulting in lower forage and fiber intake (Michael Looper 2012).

# 4.5.2. Nutrition

Cattle nutrition plays a significant role in the flavor profile of fluid milk and has been extensively researched. Feed-related flavors typically appear in fluid milk within 2 to 4 hours after a cow consumes feed, and have been explained using dairy scorecard defect terms. Feeding cows silage feeds such as alfalfa and beans had the ability to impart rank or unpleasant traits to milk. In the 1960s, the use of GC became critical for quantifying volatile chemicals that caused off-flavors in milk from certain feeds (Woods and Aurand, 2006).

In the present chapter, we have provided an overview of milk, its composition, properties, and their effects on the milk's organoleptic quality.

Another aspect that may affect the sensory characteristics of any food product is the use of different nutrition additives. Along with milk processing, conservatives, colorants,

flavors, and sweeteners surely modify milk's natural properties. In the next chapter, we will talk about a specific type of these additives; natural additives as a healthier alternative.

# Chapter 3 Natural food additives

### 1. Generalities

Food additives are natural or synthetic compounds added to foods for different purposes. The Codex Alimentarius defines a food additive as "any substance not normally consumed as a food in itself and not normally used as a characteristic ingredient of food, whether or not it has nutritive value, the intentional addition of which to food for a technological purpose in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food results, or may be reasonably expected to result, in it or its by-products becoming directly or indirectly a component of such foods." (Tomaska and Brooke-Taylor, 2014).

Additives in food preparations have been in practice since the dawn of civilization. Additives are used for several purposes; to impart or enhance flavor (taste), to give foodstuffs the desired color (look/appearance), or to increase the shelf life of the food (preservative role). Some additives are essential elements or nutritious supplements to cater for the diet deficiencies of specific groups of people (Msagati et al., 2013).

Consumers have recently become more informed about food additives and tend to prefer the additives of natural origin over their synthetic analogs (Bearth et al.,2014).

# 2. Classes of natural additives

According to the primary function of these additives, we can find antioxidants, antimicrobials, colorings, and sweeteners.

### 2.1. Natural antioxidants

Antioxidants are mostly used in food to avoid off-flavors caused by fat oxidation. There are five types of antioxidants: radical scavengers or chain-breaking antioxidants; chelators, which bind to metals and prevent them from initiating radical formation; quenchers, which deactivate high-energy oxidant species; oxygen scavengers, which remove oxygen from systems to prevent destabilization; and antioxidant regenerators, which regenerate other antioxidants when they become radicalized. Antioxidants are commonly used in meats, oils, fried foods, dressings, dairy products, baked items, and extruded snacks (Baines and Seal, 2012).

Polyphenols are one of the most interesting categories of natural substances in the vegetable kingdom. Their unique, powerful antioxidant properties have several beneficial effects on human health, including preventing cancer, osteoporosis, cataracts, cardiovascular disease, brain disease, and immunological issues (Carocho and Ferreira, 2013).

As an example of antioxidants, we can find Carnosic acid, Ferulic acid, Catechin Ascorbic acid, b-carotene, and Tocopherols (Carocho et al.,2015).

### 2.2. Natural antimicrobials

Natural antimicrobials are a prominent topic in the food industry since they ensure bacteria-free food. In theory, all natural antimicrobials have both bactericidal and fungicidal properties. A good antibacterial should be heat stable, active at low concentrations, pH unaffected, impart no flavor or color, have no toxicity, be easily assayable, resistant to contaminants, label-friendly, and cost-effective (Carocho et al.,2015).

Antimicrobials can have three resources: microorganisms, animals, and plants; antimicrobials obtained from microorganisms are substances produced by living organisms that affect others. Antimicrobials generated from plants are often secondary metabolic products that protect from predators, code for signaling molecules, and aid in stress resistance. Terpenes, steroids, alkaloids, and polyphenols are examples of chemicals produced by this process (Sun et al.,2014).

Essential oils are another major class of chemicals having antibacterial properties; complex combinations of volatile chemicals created by living organisms. The most commonly used essential oils are extracted from plants using physical methods, and they come from the mevalonate, methyl-erithrytol, and shikimic pathways, each of which produces various chemicals (Carocho et al.,2015).

### 2.3. Colorants

Colorants are added to food to make it more appealing and palatable, which are critical considerations for food. Colorants are used to enhance existing colors or to assign new ones. Natural food colorants are those that are synthesized naturally; nature-identical colorants are those that are generated in industries but mimic the natural ones, and artificial/synthetic colorants are those that are synthesized artificially (Msagati, 2013).

In the food industry, carotenoids are widely used as natural colorants; as an example, we can find capsanthin and capsorubin, B-carotene, lutein, violaxanthin, neoxanthin, b-cryptoxanthin, fucoxanthin, lycopene, and astaxanthin. Carotenoids are extracted from plants, algae, and even insects and represent a broad spectrum of colors in the food industry. The main applications of carotenoids in food are sauces, marinades, spice blends, coatings, beverages, and milk (Baines and Seal, 2012).

### 2.4. Natural sweeteners

Synthetic sweeteners have been the subject of numerous scandals and debates over the years, with claims of carcinogenicity, fœtal abnormalities, liver, bladder toxicity, and other risks necessitating the quest for natural alternatives (Carocho et al.,2015).

Natural sweeteners have the same goal as their synthetic analogs: to provide a sweet flavor while adding few or no calories to the diet; there are two kinds of natural sweeteners; Bulk sweeteners have a potency of one sucrose molecule or less (sucrose is the international standard for sweetness), and high-potency sweeteners with greater potency. To be accepted by consumers, natural sweeteners must have a good flavor, be safe, have a high solubility, high stability, and an acceptable cost (Carocho et al.,2015).

The two leading examples of this group are erythritol and tagatose. Erythritol is a sugar alcohol. It can be found in some fruits and vegetables. Tagatose is a ketohexose, an enantiomer of fructose. It can be found in very small quantities in fruits and heat-treated dairy products. It has 92% of the saccharose potential, making it very similar in taste (Carocho et al.,2015).

### 3. Plants as food additives:

Applying natural food additives extracted from plants is a sustainable development trend in the food industry. Compared with synthetic food additives, plant-based food additives have garnered considerable attention owing to their advantages (Zang et al., 2022).

Rosemary and oregano are among the most known Mediterranean plants, very used as traditional remedies and as food additives (Petter, 2004).

# 3.1. Rosemary

Rosemary (*Rosmarinus officinalis* L.) is a medicinal herb native to the Mediterranean region. It is extensively used as a condiment and food preservative in addition to its therapeutic application. *R. officinalis* L. is known for her several pharmacological activities, such as anti-inflammatory, antioxidant, antimicrobial, antiproliferative, antitumor and protective, inhibitory, and attenuating activities (Oliveira et al.,2019)



Figure 1 Rosmary « *Rosmarinus officinalis L* » ( Anonyme, 2022)

# 3.1.1. Botanical description

Rosemary belongs to the Lamiaceae family. is a dense, evergreen, hardy, perennial aromatic herb of 90–200 cm height with small (2–4 cm) pointed, sticky and hairy leaves (Fig. 16.1). The upper surface of the leaf is dark green whereas it is white below; leaves are resinous. Branches are rigid with fissured bark and stem square, woody and brown. Pale blue small flowers appear in cymose inflorescence (Petter, 2004).

Domain Eukaryota Kingdom Plantae Sub kingdom Tracheobionta **Super division** Spermatophyta Magnoliophyta Division Magnoliopsida Class Asteridae Sub class Order Lamiales **Family** Lamiaceae Genus Rosmarinus L. officinalis **Species** Binomial nomenclature Rosmarinus officinalis

Table 1Scientific classification of Rosmarinus officinalis L. (Andrade et al., 2018)

### 3.1.2. Distribution

Rosemary is native to the Mediterranean region and cultivated around the world. The cultivation of Rosemary needs a temperate climate, a dry, light, sandy soil with lots of sunlight (Petter, 2004).

### 3.1.3. Chemical composition of Rosemary

The Lamiaceae family includes plants containing large amounts of phenolic acids, terpenes, iridoids, flavonoids, and phenolic compounds (Naghibi et al.,2005).

The biologically active compounds from Rosemaryare obtained via the plant's extracts and/or essential oils. The extraction methods are applied to leaves, roots, stems, or flowers using appropriate solvents and conventional techniques like maceration, hydrodistillation, distillation, and Soxhlet by supercritical fluid extraction (Oliveira et al.,2019).

The composition of rosemary oil is 1,8-cineol (30–40%), camphor (15–25%), borneol (16–20%), bornyl acetate (up to 7%),  $\alpha$ -pinene (25%) as well as  $\beta$ -pinene, linalool, camphene, subinene, myrcene,  $\alpha$ -phellandrene,  $\alpha$ -terpinene, limonene, p-cymene, terpinolene, thujene, copalene, terpinen-4-ol,  $\alpha$ -terpineol, caryophyllene, methyl chavicol, thymol, etc. The initial distillation fraction contains mostly  $\alpha$ -thujene,  $\alpha$ -pinene, camphene,  $\beta$ -pinene and 1,8-cineol, while camphor and bornyl acetate constitute the bulk of the later distillation (Petter, 2004).

### 3.1.4. Biological activities

The demand for essential oils from medicinal plants has increased in recent years, especially in the case of oil from Rosemary, which is used as a natural food preservative (listed by the European Food Safety Authority and the US Food and Drug Administration).

Rosemary has several pharmacological activities, particularly antioxidative, antiinflammatory, antidiabetic, and antibacterial effects. These activities are related to the presence of Carnosic acid, carnosol, rosmanol, and rosmarinic acid (Oliveira and al, 2019)

# 3.1.4.1. Antibacterial activity

Rosmarinus officinalis L. is widely used today as a food preservative because of its powerful antibacterial activity. These properties are mediated via several mechanisms; it disrupts the permeability barrier in cells and induces various morphological and physiological changes. Its lipophilic moieties attack the cytoplasmic membrane, leading to changes in stability, hydrophobicity, fluidity, and fatty acid composition. They also disrupt proton pump function, destabilizing cell membrane architecture, which causes an uncontrolled flux of H+ ions, resulting in the inhibition of H+ ion-dependent movement of solutes across the membrane and the disruption of the intracellular pH (Kordali et al., 2005).

Rosemary extracts can effectively inhibit the reproduction of many microorganisms such as *Escherichia coli, Staphylococcus aureus, Candida albicans, Saccharomyces cerevisiae, S. aureus*, and *methicillin-resistant S. aureus* (Ekambaram et al.,2016).

### 3.1.4.2. Antioxidant activity

Rosemary extracts contain a variety of antioxidant components such as rosmarinic acid, sagenoic acid, and carnosol, which is a natural non-toxic new antioxidant. Rosmarinic acid and lipid peroxide block lipid peroxidation by competitive binding; can also promote the recovery of mitochondrial membrane potential by inhibiting the formation of reactive oxygen species in cells and reducing the activation of c-Jun N-terminal kinase and extracellular signal-regulated kinase (Dai and Liu, 2021).

It was found that the antioxidant activity of rosmarinic acid was closely related to its structure. The O-diphenol hydroxyl in the structure could scavenge free radicals so that rosmarinic acid entered the lipid bilayer and played an antioxidant role. Studies have confirmed that carnosic acid of rosemary extracts can interact with each component, and

when scavenging reactive oxygen, carnosic acid can produce secondary antioxidants. This process may enhance antioxidants' activity. In addition, the combination of ursolic acid and oleanolic acid in rosemary extracts also has an antioxidant effect to a certain extent (Loussouarn et al.,2017)

# 3.1.4.3. Antitumor activity

Studies have found that carnosol increased significantly the ratio of estrogen receptor  $\alpha$  and  $\beta$  subtypes, preventing the proliferation of breast cancer cells and blocking them in the S phase. In addition, scientists have confirmed that rosmarinic acid could also inhibit the proliferation and differentiation of breast cancer cells MDA-MB-231 and could even induce their apoptosis and reduce migration (Bo-xue et al.,2018).

# 3.2. Oregano

Etymologically, the name Origanum comes from the plant's Greek name (origanon), which derives from the words (oros = mountain) and (ganos = brightness, beauty), since Oregano grows at altitudes of 400-1,800 m and in sunny places (Villalobos-Pascual and Acosta-Ballesta, 2003).

Oregano, *Origanum vulgare*, also called origanum or wild marjoram, is an aromatic perennial herb of the mint family Lamiaceae known for its flavourful dried leaves and flowering tops. The herb has long been an essential ingredient of Mediterranean cooking and is widely used to season many foods. Culinary varieties, such as Greek or Italian Oregano, have a strong aroma and a warm, pungent taste (Petter, 2004).



Figure 2 Oregano, (Origanum vulgare) (anonyme, 2022)

# 3.2.1. Botanical description

Oregano is generally considered as a perennial herb, with creeping roots, branched woody stems, and opposite, petiolate and hairy leaves. The flowers are in corymbs with reddish bracts, a two-lipped pale purple corolla, and a five-toothed calyx. In moderate climates, the flowering period extends from late June to August. Each flower produces, when mature, four small seed-like structures. The foliage is dotted with small glands containing the volatile or essential oil that gives the plant its aroma and flavour (Petter, 2004).

Table 2 Scientific classification of Oregano (National Plant Germplasm System, 2022)

Domain	Eukaryota
Kingdom	Plantae
Sub kingdom	Tracheobionta
Super division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Sub class	Asteridae
Order	Lamiales
Family	Lamiaceae
Genus	Origanum
Species	O. vulgare
Binomial nomenclature	Origanum vulgare

### 3.2.2. Origin and distribution of Oregano

The members of the genus are mainly distributed around the Mediterranean region: 35 out of 43 occur in the East Mediterranean, exclusively; four species are found restricted in the West Mediterranean, while three are endemic to Libya. In addition, hybrids that have been found when Origanum species co-occur, either in natural or in artificial conditions (Petter, 2004).

### 3.2.3. Chemical compositions

Although abundant chemical compounds have been isolated from oregano, the most important group, from a commercial and application point of view, refers to its volatile oils, basically composed of terpenoids. However, composition may vary significantly among different genotypes. Oregano species are rich in phenolic monoterpenoids such as carvacrol, while species rich in bicyclic monoterpenoids cis- and trans-sabinene hydrate are commercially designated as marjoram. It is quite easy to distinguish the difference between the pungent smell of oregano and the sweet smell of marjoram. In the first group are a number of chemically related compounds such as γ-terpinene p-cymene, thymol and carvacrol methyl ethers, thymol and carvacrol acetates; also compounds such as p-cymenene, p-cymen-8-ol, pcymen-7-ol, thymoquinone, and thymohydroquinone are also present. In the second group, α-thujene, sabinene, cis- and trans-sabinene hydrate acetates, cis- and trans-sabinol, and sabina ketone can also be found Other chemical groups that are commonly detected in Origanum species are acyclic monoterpenoids such as geraniol, geranyl acetate, linalool, linalyl acetate, and β-myrcene; bornane-type compounds such as camphene, camphor, borneol, and bornyl and isobornyl acetate; and sesquiterpenoids, such as  $\beta$ -caryophyllene,  $\beta$ bisabolene, β-bourbonene, germacrene-D, bicyclogermacrene, α-humulene, α-muurolene, γallo-aromadendrene, muurolene. γ-cadinene, α-cubebene, α-copaene, α-cadinol, coryophyllene oxide and germacrene-D-4-ol. (Petter, 2004).

# 3.2.4. Biological activities

### 3.2.4.1. Antioxidant activities

Oregano's essential oil has an intense antioxidant power with remarkable effects in preventing fat oxidation due to its high content of thymol and carvacrol. However, this essential oil as a food preservative is rather limited because of its strong smell, which negatively affects the food's organoleptic properties (Lambert and al ,2001).

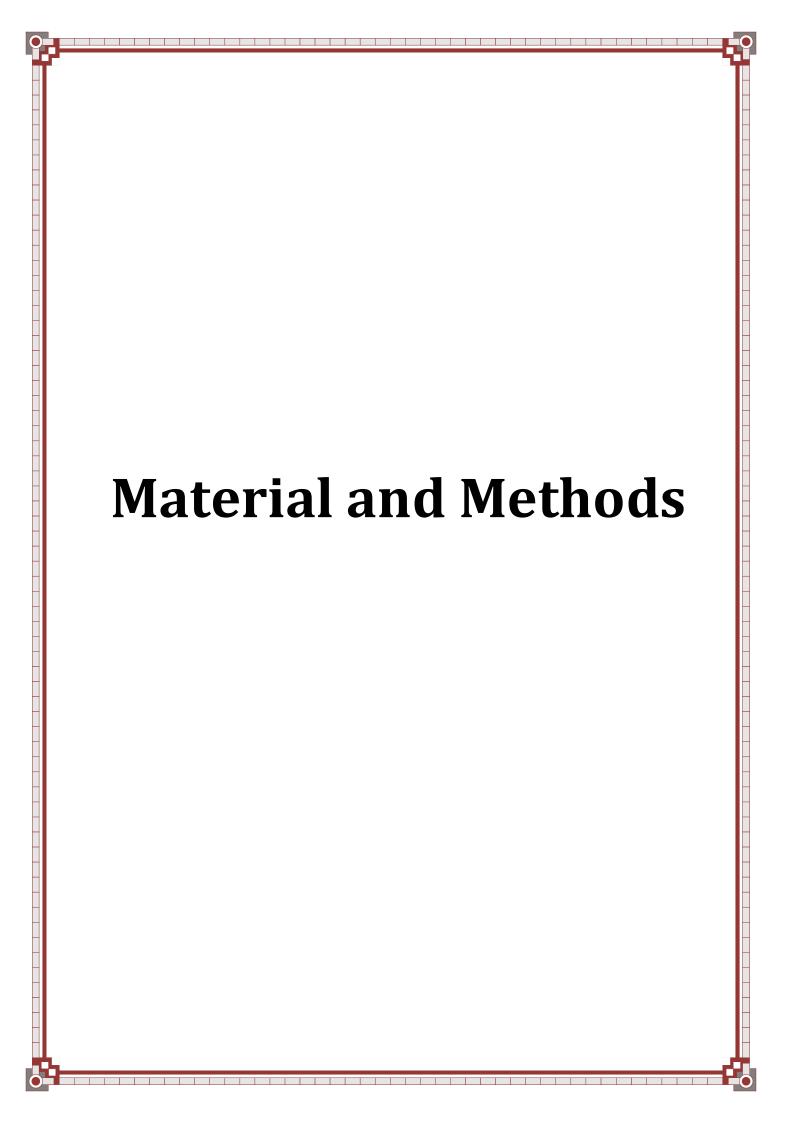
Researches reveal that essential oils from the different parts of O. vulgare L. have excellent antioxidant activities due to its contents of carvacrol and thymol (Han and al; 2017).

# 3.2.4.2. Antibacterial effects

The oregano essential oils have an interesting antibacterial effect associated with their phenolic components, carvacrol, and thymol. These properties are often used against gram-negative and gram-positive bacteria like *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus ,and Clostridium perfringens* (Lambert and al ,2001).

In this present literature review, we have attempted to be exhaustive in gathering all theoretical knowledge required for a better understanding of the subject. We started with some generalities about sensory evaluation with a brief explanation of their different methods. We then moved on to milk and different factors affecting its sensory properties before getting into the topic of natural additives and their biological activities as healthier substitutes to their synthetic analogs.

# **Application part**



### Material and methods

This study analyses the sensory effect of adding Rosemary and Oregano aqueous extracts to natural raw milk. The data are obtained from a previous study of master's students done by Lekhnafer and Medjdoub (2021) entitled "Sensory analysis of raw milk supplemented with natural extracts".

Our work's add value consists of a thorough sensory evaluation using statistical tests and data analysis methods.

# 1. The experimental origin of the data

Before the biomathemathical analysis, it is important to summarize first the experimental phase done previously providing the source of data used in the present study. This phase passes through five different steps:

- Collection of rosemary and oregano leaves: The collection was from two regions of MILA: Marachou and Boufouh.
- Preparation of the aqueous extracts: after drying, Rosemary's and Oreganos' leaves, aqueous solutions with a 1 mg/ml concentration for Rosemary and 5 mg/ml for Oregano were prepared.
- Collection of raw milk: the collection was from three different farms in Sidi Merouane, Mila.
- Adding extracts to raw milk with two concentrations for each extract: 1mg/ml and 0.75mg/ml for rosemary, and 5 mg/ml and 1.25 mg/ml for oregano.
- A sensory evaluation for raw milk was performed with 51 tasters with a studied protocol.

### 2. Biomathematical analysis of the Sensory data

In the present study, we are working on a hedonic test to determine the level of satisfaction with milk after adding natural extracts.

We have used a five-level categorical scale ranging from extremely unpleasant to extremely pleasant, passing through unpleasant, neutral, and pleasant.

Tasters chose the category that best matches their level of satisfaction based on four characteristics that describe the sensation: color, odor, texture, and flavor. The following tables recapitulate data resulting from the sensory evaluation performed with 51 tasters.

**Table 1.** Recapitulative data of the sensory evaluation for the control sample.

	Control sample			
	color	odor	flavor	texture
Very pleasant	15	13	14	17
	29%	25%	27%	33%
Pleasant	34	30	34	29
	67%	59%	67%	57%
Neutral	1	1	0	4
	2%	2%	0%	8%
Unpleasant	1	5	2	1
	2%	10%	4%	2%
very	0	2	1	0
unpleasant	0%	4%	2%	0%
Total	51	51	51	51
	100%	100%	100%	100%

Table 2:Recapitulative data of the sensory evaluation for the rosemary samples

Rosmarinus		Rosmarinus		75%	R	osmarinu	ıs	100%
	R_C_0,75	R_O_0,75	R_F_0,75	R_T_0,75	R_C_1	R_O_1	R_F_1	R_T_1
Very pleasant	10	11	13	15	14	10	7	11
	20%	22%	25%	29%	27%	20%	14%	22%
Pleasant	28	24	18	30	23	24	30	34
	55%	47%	35%	59%	45%	47%	59%	67%
Neutral	1	2	3	3	2	3	3	1
	2%	4%	6%	6%	4%	6%	6%	2%
Unpleasant	10	13	14	2	10	13	7	4
	20%	25%	27%	4%	20%	25%	14%	8%
very	2	1	3	1	2	1	4	1
unpleasant	4%	2%	6%	2%	4%	2%	8%	2%
Total	51	51	51	51	51	51	51	51
	100%	100%	100%	100%	100%	100%	100%	100%

# With:

- R C 1,25 The variable color for the sample of milk supplemented with rosemary 0,75 g/L;
- R O 1,25 The variable odor for the sample of milk supplemented with rosemary 0,75 g/L;
- R F 1,25 The variable flavor for the sample of milk supplemented with rosemary 0,75 g/L;
- R\_T\_1,25 The variable texture for the sample of milk supplemented with rosemary 0,75 g/L;
- R C 5 The variable color for the sample of milk supplemented with rosemary 1 g/L;
- R\_O\_5 The variable odor for the sample of milk supplemented with rosemary 1 g/L;
- R F 5 The variable flavor for the sample of milk supplemented with rosemary 1 g/L;
- R T 5 The variable texture for the sample of milk supplemented with rosemary 1 g/L;

25% 100% Origanum Origanum Origanum 0\_0\_1,25 O\_C\_5 O\_C\_1,25 O\_F\_1,25 O\_T\_1,25 0\_0\_5 O\_F\_5 O T 5 Very 5 5 9 8 7 4 2 7 pleasant 14% 10% 10% 14% 8% 18% 4% 16% Pleasant 17 25 22 27 30 17 17 16 43% 53% 33% 59% 33% 33% 31% 49% Neutral 3 2 4 0 2 1 4 1 2% 6% 4% 8% 0% 4% 2% 8% Unpleasant 12 19 10 16 18 6 9 17 31% 24% 35% 12% 18% 33% 37% 20% very 5 9 4 16 13 4 4 11 unpleasant 10% 8% 18% 8% 31% 22% 25% 8% Total 51 51 51 51 51 51 51 51 100% 100% 100% 100% 100% 100% 100% 100%

Table 3: Recapitulative data of the sensory evaluation for the oregano samples

### With:

- O C 1,25 The variable color for the sample of milk supplemented with oregano 1,25 g/L;
- O O 1,25 The variable odor for the sample of milk supplemented with oregano 1,25 g/L;
- O\_F\_1,25 The variable flavor for the sample of milk supplemented with oregano 1,25 g/L;
- O T 1,25 The variable texture for the sample of milk supplemented with oregano 1,25 g/L;
- O C 5 The variable color for the sample of milk supplemented with oregano 5 g/L;
- O\_O\_5 The variable odor for the sample of milk supplemented with oregano 5 g/L;
- O F 5 The variable flavor for the sample of milk supplemented with oregano 5 g/L;
- O T 5 The variable texture for the sample of milk supplemented with oregano 5 g/L;

# 3. Data analysis

# 3.1 Scaling

Our expirement uses a hedonic scale with five different intervalles, so before any manipulation, this interval categories must be converted into numerical notations ranging from 1 to 5, with "1" corresponding to extremely unpleasant, "2" to unpleasant, "3" to neutral, "4" to pleasant, and "5" to extremely pleasant.

### 3.2 Radar chart

Once we have converted our data to numerical, we can now calculate the averages of each attribute variable and realize the radar chart.

	Color	Odor	Flavor	Texture
Control	4,24	3,92	4,14	4,22
Rosemary 0,75 g/L	3,67	3,61	3,47	4,10
Rosemary 1g/L	3,73	3,57	3,57	3,98
Oregano 1,25g/L	3,20	3,33	2,82	3,59
Oregano 5 g/L	2,88	2,73	2,51	3,45

Table 4:The average of sensory variables of different groups

For example, to calculate the mean of the control group for the variable color, we can see in (table 1) that this variable has for apprecaitions: 15 very pleasant "5", 34 pleasant "4", 1 neutral "3", 1 unpleasant "2", and 0 very unpleasant "1". So, the average is calculated as follow: Color average for the control sample =  $\frac{15 \times 5 + 34 \times 4 + 1 \times 3 + 1 \times 2 + 0 \times 1}{51} = 4,24$ 

The same way, we calculate the other means. The radar chart is a graphical method of displaying multivariate data in a two-dimensional chart of three or more quantitative variables, four in our case, represented on axes starting from the same point.

We can use Microsoft Excel 2010 to realize radar chard, a spreadsheet developed by Microsoft. It features calculation or computation capabilities, graphing tools, pivot tables, and a macro programming language called Visual Basic for Applications.

After selecting our data in the spreadsheet, in the menu **Insert**, we choose **Other charts** from the category **Charts**, then we click on **Radar chart** (figure 1).

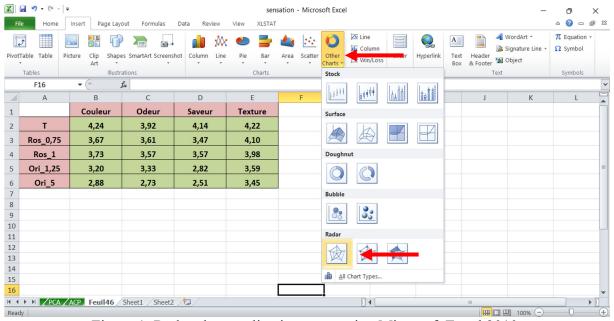


Figure 1: Radar chart realization steps using Microsoft Excel 2010.

# 3.3 Principal Components Analysis

The PCA method is used to detect any eventual correlation patterns among sensory attributes.

To apply PCA, IBM SPSS statistics version 28 was used, a software developed by IBM that enables sophisticated statistical analysis for data management and advanced multivariate analysis.

In the menu Analyze of SPSS, we choose Dimension reduction then Factors (figure2).

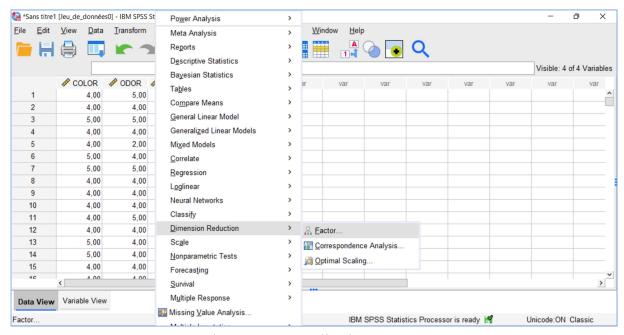


Figure 2: PCA realization 1st step

The next dialogue box appears, we choose our variables then we make the following changes (figure 3):

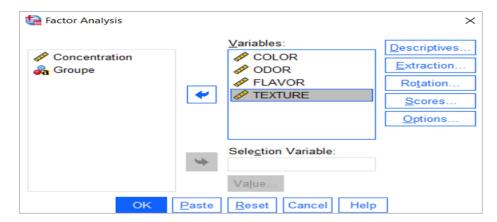


Figure 3: PCA realization 2nd step

- In the menu **Descriptives**, we check **Univariate descriptive** in statistics and coefficients in correlation Matrix;
- In the menu Extraction, we check **Scree plot**;
- In the menu Rotation, we check Display loading plot;
- In the menu Scores, check Display factor score coefficient matrix;
- And finally in the menu **Options**, check **Replace missing values with the mean** then we click on Ok.

# 3.4 The overall acceptance

To evaluate the overall acceptance of milk by tasters, we will calculate a weighted average of the attribute variables scores. As weights, we will use the IBS scorecard:

Attribute Perfect score

Odor 20

Flavor 40

Body (Texture) 30

Color and appearance 10

Table 5: IBS Milk score card scores

The overall score will be calculated using the formula:

$$Overall\ score = \sum Attributes' notation \times \frac{score}{5}$$

$$Overall\ score = \frac{Odor\ notation \times 20 + Flavor\ notation \times 40 + Texture\ notation \times 30 + Color\ notation \times 10}{5}$$

Once we have the overall scores for the control group, and after adding the extracts with different concentrations, we can make comparisons between different groups and different attribute variables.

### 3.5 Mean comparison tests

To determine if there is a significant difference between means of different groups or attribute variables, we must conduct a mean comparison test; this test could be a strong parametric test or a robust non-parametric test.

To apply the parametric test, t-student in our case, we have two assumptions to verify:

- Normality of the distributions of the variables;
- Homogeneity of the variances.

If one of these two conditions is not verified, we apply the non-parametric test, which is more robust but not as strong as the parametric test.

### 3.5.1 Verification of the conditions

The central limit theorem states that if a population with mean  $\mu$  and standard deviation  $\sigma$  and takes sufficiently large random samples from the population, then the sample will be approximately normally distributed.

However, to be more accurate, we find it necessary to perform a normality test for the distributions of our samples together with the test of homogeneity of variance.

Using IBM SPSS version 28, we can perform the two tests together; in the menu Analyze, we choose **Descriptive statistics**, and then **explore** (figur4).

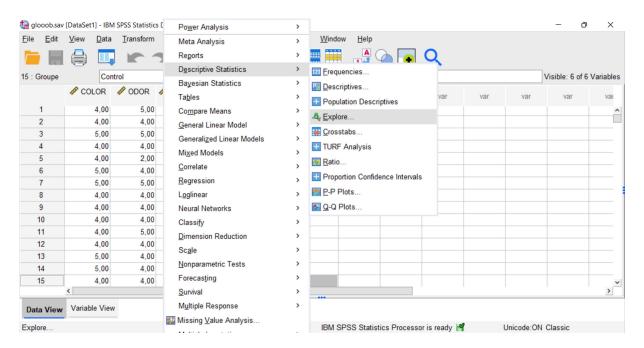


Figure 4: realizing normality and homogeniety of variance 1st step

A dialogue box appars; we check **Normality plots with tests** in the **Boxplot** tab and **Untransformed** in the **Spread vs level with Levene test tab** (figure 5).

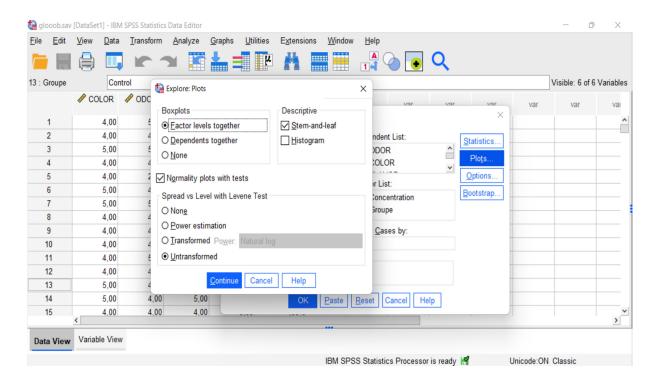


Figure 5: realizing normality and homogeniety of variance 2nd step

We have performed these tests for the overall scores samples and for each attribute variable using the concentration and the type of the extract as factors.

The following tables recapitulate the results of our test:

# 3.5.2 Normality tests:

As a normality test, we are going to perform Shapiro-Wilk test.

The following tables illustrate the results of the Shapiro-Wilk test of normality for both overall scores and attribute variables scores

	Statistic	df	Sig.
Control	0,827	51	0,000
Rosemary_0.75 mg/ml	0,952	51	0,037
Rosemary_1mg/ml	0,946	51	0,021
Oregano_1.25mg/ml	0,929	51	0,005
Oregano_5mg/ml	0,883	51	0,000

Table 6: Shapiro-Wilk test results for overall scores

Table 7: Shapiro-Wilk test results for attributes variables scores

	Sl	napiro-Wilk	
	Statistic	df	Sig.
C_C	0,682	51	0,000
C_0	0,742	51	0,000
C_F	0,649	51	0,000
C_T	0,773	51	0,000
R_C_0.75	0,791	51	0,000
R_O_0.75	0,817	51	0,000
R_F_0.75	0,849	51	0,000
R_T_0.75	0,743	51	0,000
R_C_1	0,817	51	0,000
R_O_1	0,827	51	0,000
R_F_1	0,780	51	0,000
R_T_1	0,700	51	0,000
O_C_1.25	0,839	51	0,000
0_0_1.25	0,816	51	0,000
O_F_1.25	0,854	51	0,000
O_T_1.25	0,781	51	0,000
O_C_5	0,811	51	0,000
0_0_5	0,849	51	0,000
O_F_5	0,825	51	0,000
O_T_5	0,838	51	0,000

# With:

 $C_C$ : The variable "color" for the control sample; C O: The variable "odor" for the control sample; C\_F: The variable "flavor" for the control sample;  $C_T$ : The variable "texture" for the control sample;  $R_C_{0.75}$ : The variable "color" for the rosemary sample with 0,75g/L;  $R_O_{0.75}$ : The variable "odor" for the rosemary sample with 0,75g/L; R F 0.75: The variable "flavor" for the rosemary sample with 0,75g/L; R T 0.75: The variable "texture" for the rosemary sample with 0,75g/L; R C 1: The variable "color" for the rosemary sample with 1g/L; R O 1: The variable "odor" for the rosemary sample with 1g/L;  $R_F_1:$ The variable "flavor" for the rosemary sample with 1g/L; The variable "texture" for the rosemary sample with 1g/L;  $R_T_1:$ O\_C\_1.25: The variable "color" for the oregano sample with 1,25g/L; O O 1.25: The variable "odor" for the oregano sample with 1,25g/L; O F 1.25: The variable "flavor" for the oregano sample with 1,25g/L; O T 1.25: The variable "texture" for the oregano sample with 1,25g/L; O\_C\_5: The variable "color" for the oregano sample with 5 g/L; 0 0 5: The variable "odor" for the oregano sample with 5 g/L; O\_F\_5: The variable "flavor" for the oregano sample with 5 g/L; O T 5: The variable "texture" for the oregano sample with 5 g/L;

The signification coefficients of all samples are under 0.05, so we reject the null hypothesis  $H_0$ . All the samples have a non-normal distribution; the first condition is not verified.

# 3.5.3 Homogeneity of variance

To test homogeneity of variance we have used Levene's test. Levene's test (Levene 1960) is used to test if k samples have equal variances. Equal variances across samples is called homogeneity of variance.

The following tables illustrate the results of levene's test of homogeneity of variance for both overall scores and attributes variables scores

Factor		Levene Statistic	df1	df2	Sig.
Concentration	Overall_score	21,214	2	252	0,000
Group	Overall_score	13,142	3	251	0,000
Concentration	odor	9,621	3	251	0,000
	color	28,712	3	251	0,000
	flavor	24,507	3	251	0,000
	texture	4,671	3	251	0,003
Group	odor	13,947	2	252	0,000
	color	44,759	2	252	0,000
	flavor	26,815	2	252	0,000
	texture	16,041	2	252	0,000

Table 8: Levene's test results for overall scores and attributes variables scores

The signification coefficients of all samples are under 0.05, so we reject the null hypothesis  $H_0$ . The variances of the samples are different. The second condition is also not verified.

Since the two conditions are not verified, we cannot use the parametric test of Student. So, we will use a non-parametric alternative, the test of Wilcoxon.

The Wilcoxon test compares **two paired groups**. The goal of the test is to determine if two or more sets of pairs are different from one another in a statistically significant manner.

We can perform this test using IBM SPSS Statistics software. In the menu Analyze of SPSS, we choose Non-parametric tests, and then Related samples.

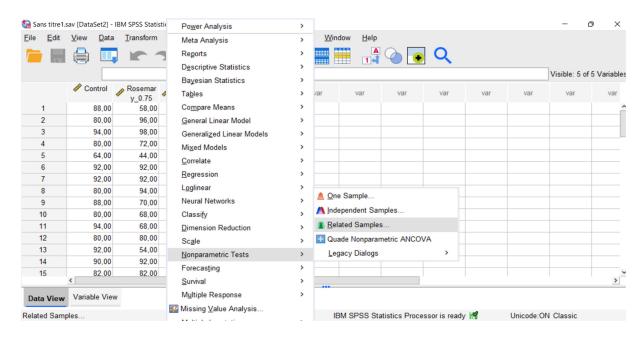


Figure 6: Realization of Wilcoxon test 1st step

A dialogue box with three tabs appears. We check **Customize analysis** in the **Objective** tab, we choose the two variables to compare in the **Fields** tab, and we check The **Wilcoxon test** in the **Settings** tab. then we click on **Run**.

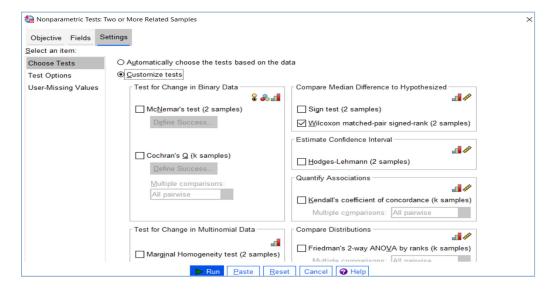
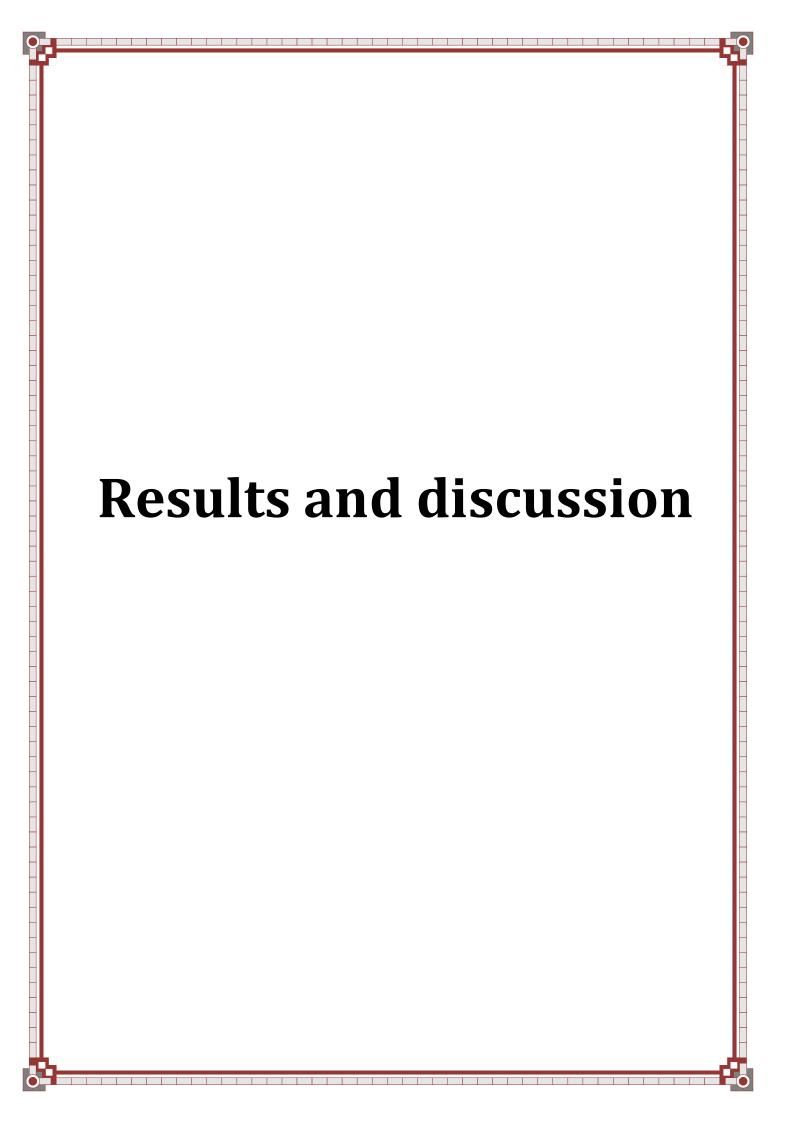


Figure 7: Realization of Wilcoxon test 2nd step

This present section was devoted to discuss our case study's methodology. We've began by summarizing the expirement used to collect needed sensory data, then we've presented different methods used in their analysis using different softwares. In the next section, we will try to interpret and analyse our results in order to provide an answer to our problematic.



### Results and discussion

In the present chapter, we will expose the results analysis and try to interpret each result individually. Overall scores will be attributed to different samples and then dissected to point out the variables responsible for the observed differences between sensory qualities.

### 1. Results

### 1.1. Radar chart

The radar chart (figure 1) allows us to realize a basic visual comparison between raw milk, milk with rosemary extracts, and milk with oregano extracts. This comparison is multidimensional because it takes into consideration the four variables: color, odor, flavor, and texture.

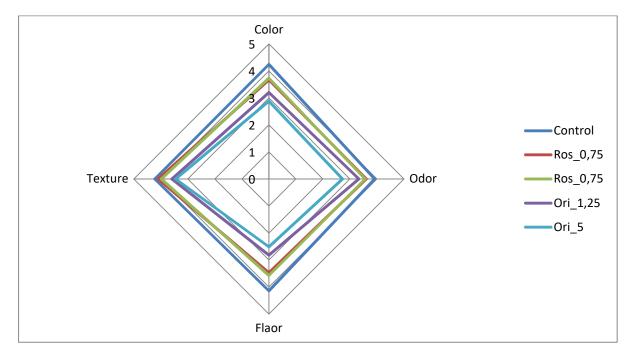


Figure 1: Radar chart representing sensory variables (Color, odor, flavor and texture) for oregano (1.25g/l and 5.00g/L) and Rosemary (0.75g/l and 1g/L)

The plot shows that the control sample of raw milk has higher ratings for all the variables followed by rosemary samples, then, in the last position, oregano samples.

We can see that the variable texture has the highest appreciations for all the samples with the minimum differences while flavor has the lowest.

# 1.2. Principal components analysis:

Varia

**Cumulative %** 

Before any representation with principal components, we should first choose the components to use and their interpretation. To do so, we will analyze (table 1) of the correlation between variables and components and table 2 of the variance by component.

	F1	F2	F3	F4
COLOR	0,808	-0,291	-0,368	-0,357
ODOR	0,839	-0,163	-0,136	0,502
FLAVOR	0,806	-0,152	0,559	-0,119
TEXTURE	0,669	0,739	-0,059	-0,055

Table 1: Correlations between variables and components

In our results, the first principal component has large positive associations with all the variables, so it primarily measures an overall sensory quality; it allows an acceptable evaluation with 61,32% of the model information with only one dimension.

The second component has large positive associations with the variable texture, so it primarily measures mouth-feel. The third component has large positive association with flavor. The fourth component has large positive association with odor and a negative association with color, so it primarily measures the variables of the first interaction.

	F1	F2	F3	F4
bility (%)	61,320	17,019	11,758	9,903

90,097

100,000

78,339

61,320

Table2: The variance explained by component

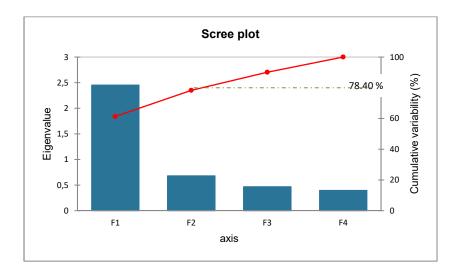


Figure 2:Variance explained by component

Using the cumulative proportion of the amount of variance explained, we decided to retain the first two principal components that explain an acceptable level of 78.40 % of the variance.

To interpret each principal component, we examine the magnitude and direction of the coefficients for the original variables (Figure 3).

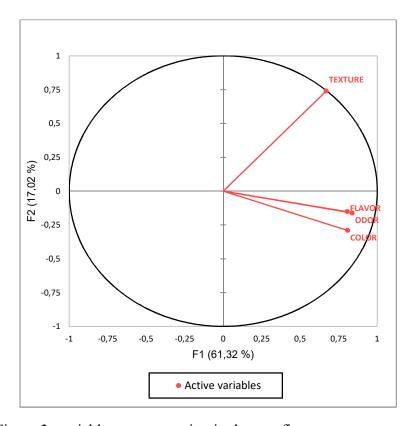


Figure 3: variables representation in the two first components axes

The plot shows the results for the first two components; we can see that the variables texture, color, flavor, and odor are all close to the correlation circle and hence well represented using the PCA (with the first two components). The two variables, flavor and odor, are superposed, which indicates a very strong correlation. These two variables form an acute angle with the variable color, which means that they correlate reasonably well. On the other hand, the almost right angle between color and texture indicates that these two variables are independent.

The strong correlation between odor and flavor can be explained by the fact that there are common stimuli for these two related attributes; volatile substances that stimulate both taste and olfactory senses.

The correlation between these two related variables and the variable color is due to the presence of flavonoids and tannins responsible for aromatic, flavoring, and coloring properties.

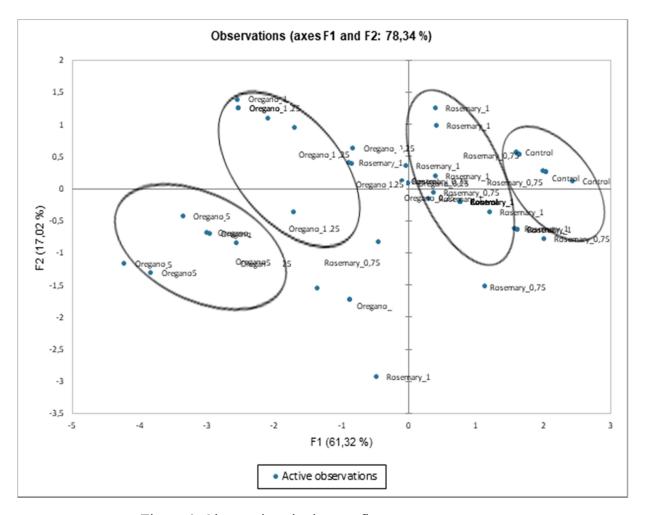


Figure 4: Observations in the two first components axes

The observation plot allows us to visually order our samples according to the first component, representing an overall sensory quality with 61% accuracy. We can notice that milk samples with oregano extracts are mostly on the left of the first axe, indicating a low sensory quality value, followed by milk with rosemary with an acceptable quality very close to the control group with the highest sensory quality.

As for the second component, we notice slight differences between samples, suggesting that the extracts didn't much affect the variable texture (as indicated in the radar chart above).

### 1.3. The overall acceptance

These overall scores are calculated based on weights representing each attribute's importance.

The following table shows the average and the standard deviation of each sample:

	Control	Rosemary 0,75g/L	Rosemary 1g/L	Oregano 1,25g/L	Oregano 5g/L
average	81,57	72,55	72,82	63,57	55,29
Stddeviation	13,73	17,42	16,18	19,50	23,68

Table 3: averages and standard deviations of milk samples

Clearly, raw milk with no additives has the biggest score of **81,57/100** with the lowest standard deviation of **13,74**, which means that all the tasters have provided close or similar appreciations.

Milk with Rosemary 1g/L extract scored the second with **72,82/100** and a standard deviation of **16,18**, which means that the tasters have close appreciations. Milk with rosemary **0,75g/L** extract has a very close score of **72,55/100** with an acceptable standard deviation of **17,42**.

Milk with Oregano extracts scored the worst with 63,57/100 and 55, 29/100 for the concentrations of 1,25g/L and 5g/L, respectively. The standard deviations are also high (19,50, and 23,68), which means that the appreciations were very dispersed.

To approve these results statistically, we have performed the test of Wilcoxon; the next table shows the results:

	Control	Rosemary 0,75g/L	Rosemary 1g/L	Oregano 1,25g/L	Oregano 5g/L
Control	1	0,006	0,003	0,000	0,000
Rosemary 0,75g/L	/	/	0,838*	0,000	0,000
Rosemary 1g/L	/	/	/	0,000	0,000
Oregano 1,25g/L	/	/	/	/	0,000
Oregano 5g/L	/	/	/	/	/

Table 4:wilcoxon's test results

There is only one significant value of **0,838** between Rosemary **0,75g/L** milk sample and Rosemary **1g/L** milk sample, so we retrain the null hypothesis: the samples are considered similar.

All the other comparisons have a signification value under 0,05, so we reject the null hypothesis; the samples observed are statistically different.

So, these results approve the order we attributed to the sensory qualities; Raw milk has the highest sensory quality, followed by Rosmarinus, then oreganos in last place.

Based on the results of the oregano extracts, we can say that additive concentration negatively correlates with sensory scores.

### 1.4. Difference tests:

To this point, all the analyses have more of a global perspective. In this part of the results, we will dissect the scores and point out the variables responsible for the differences between sensory qualities for each sample.

The following table summarizes the results of the Wilcoxon test for each attribute variable between raw milk and milk with rosemary and oregano extracts:

Table 5: Wilcoxon test's results( comparison between Rosemary and Oregano)

	Rosemarinus	Oregano
Color	0,007	0,000
Odor	0,006	0,000
Flavor	0,004	0,000
Texture	0,051	0,000

When the p-value is under 0.05, we reject the null hypothesis, which means that there is a significant difference in sensory quality, while a p-value above 0.05 means that there is no significant difference, so the samples are considered similar.

On the one hand, we notice a difference in three variables: color and flavor, and odor which means that adding rosemary extracts only affects these three sensory variables.

On the other hand, the tests on oregano extracts show significant differences for all the variables, so adding oregano modifies all the sensory variables.

# 2. Discussion:

Adding *Rosemarinus* and *Oreganum* extracts has affected the sensory quality of raw milk but in different ways. Milk with rosemary has an acceptable quality score of 72/100 for the two studied concentrations, while milk with oregano scores 63,57/100 and 55,29/100 for the concentrations of 1,25g/L and 5g/L, respectively.

When we searched deeper for the variables responsible for these significant differences, we discovered that Rosemary extracts affect only three of the four variables: color, odor and flavor. On the other hand, adding oregano seems to affect all the sensory variables, which explains the dramatic drop in the overall sensory quality score.

The observed correlations between the different sensory variables are related to the chemical composition of the extracts; a correlation between variables, for example, is due to common responsible chemical components, while the independence of variables is due to different ones.

Following this logic, the strong correlation between odor and flavor can be explained by the fact that there are common stimuli for these two related attributes; by definition, flavor includes olfactory perceptions caused by volatile substances released from a product in the mouth via the posterior nares. In our case, different volatile compounds like cineole, p-cymene, linalool, gamma-terpinene in rosemary, carvacrol, thymol and  $\gamma$ -terpinene in oregano stimulate both taste and olfactory senses, resulting in parallel modifications in both flavor and odor variables.

The correlation between these two related variables and the variable color is due to the presence of flavonoids and tannins responsible for aromatic, flavoring, and coloring properties. Tannins are known for their yellowish white to brown color and astringent taste, while flavonoids are known for their color ranging from light yellow to golden and for their bitter, acrid, and astringent taste.

As for the effect of the concentration, which is statistically evident in the case of oregano, it is negatively correlated with the sensory quality. The use of natural extracts must, then, be in an interval of concentration that guarantees a certain balance allowing to realize the desired effects without much affecting sensory quality.

The comparison between the qualities of milk supplemented with the two extracts shows higher scores in favor of Rosmarinus, which can be explained by the use of different concentrations; oregano was used with bigger concentration which affect negatively its sample sensory qualities (5g/L and 1,25 g/L for oregano and 1g/L and 0,75g/L for rosemary). The difference can also be related to the chemical composition and the concentrations of components; Oregano is known for its high concentrations in phenolic compounds (more than 78.85% in oregano essential oil, primarily carvacrol and thymol) in comparison to rosemary, with terpenes as major component; (1,8-cineol (30–40%), camphor (15–25%), borneol (16–20%), bornyl acetate (up to 7%), α-pinene (25%) (Petter, 2004).

From another perspective, Garine (1972) has proven that sociocultural considerations also impact the acceptability of food in general. Individuals are predisposed to accept traditional plants widely used in their region. The perception of these plants seems familiar and natural, so it does not affect much sensory quality of the food they're used as additives and can even ameliorate the sensory quality in certain cases.

Rosemary is a well-known plant in the region of our study, it's widely cultivated as an ornamental plant and used in food as a spice and for its medicinal effects. This fact may have played in its favor in evaluating sensory quality. The use of oregano, on the contrary, is not common although it's native to the region; for that reason, its sensory perception seems less familiar and hence low evaluated.

# Conclusion

#### **Conclusion**

Our present study aims to analyze the sensory effect of milk supplementation by aqueous extracts of rosemary and oregano with different concentrations. The results of our analysis allow us to evaluate the sensory quality of different samples. Milk with rosemary has an acceptable quality score of 72/100 for the two studied concentrations, while milk with oregano scores 63,57/100 and 55,29/100 for the concentrations of 1,25g/L and 5g/L, respectively.

The difference between samples is explained by the fact that the aqueous extracts affect sensory variables differently, which can be related to their chemical composition. As for the concentration, it is proven to affect the sensory quality negatively; the use of natural extracts must, then, be in an interval of concentration that guarantees a certain balance allowing to realize the desired effects without much affecting sensory variables.

Once affined, this study can provide practical solutions for two of the main problems facing the use of natural extracts as substitutes for synthetic food additives: the choice of the extract to use and the optimal concentration.

As a perspective, we find it interesting to realize a combined study with chemical, physical, microbiological and sensory analysis. For more precision, we can use different concentrations and try to choose the one that realizes the optimal combination of all the studied aspects.

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#### Abstract

Sensory evaluation is a primordial phase in developing new food products; it allows analyzing sensory properties and understanding the factors controlling the consumer's acceptance. The present study analyses, with various biomathematical methods, the effect of milk supplementation by aqueous extracts of rosemary and oregano at different concentrations. The results of this multivariable analysis allow the evaluation of the sensory quality of different samples and optimize the concentrations of plant extracts supplementation in respect to milk sensory quality.

Key words: Milk, plants extracts, sensory analysis, radar chart, PCA, score

#### ملخص

التقييم الحسي هو مرحلة أساسية في تطوير منتجات جديدة, يسمح بتحليل الخصائص الحسية وفهم العوامل التي تتحكم في قبول المستهلك. تحلل الدراسة الحالية تأثير مكملات الحليب بالمستخلصات المائية للإكليل والأوريجانو بتركيزات مختلفة بطرق رياضية حيوية مختلفة. تسمح نتائج هذا التحليل متعدد المتغيرات بتقييم الجودة الحسية للعينات المختلفة وتحسين تركيزات مكملات المستخلصات النباتية فيما يتعلق بالجودة الحسية للحليب.

الكلمات المفتاحية: حليب ، مستخلصات نباتية ، تحليل حسي ، مخطط رادار ، التحليل عبر المركبات الرئيسية ،تقييم.

#### Résumé

L'évaluation sensorielle est une phase primordiale dans le développement de nouveaux produits alimentaires; elle permet d'analyser les propriétés sensorielles et de comprendre les facteurs contrôlant l'acceptation du consommateur. La présente étude analyse avec diverses méthodes biomathématiques, l'effet de la supplémentation du lait par des extraits aqueux de romarin et d'origan à différentes concentrations. Les résultats de cette analyse multivariée permettent d'évaluer la qualité sensorielle de différents échantillons et d'optimiser les concentrations de supplémentation en extraits de plantes afin d'améliorer la qualité sensorielle du lait.

Mots clés: Lait, extraits de plantes, analyse sensorielle, carte radar, PCA, score