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MASTER

Thème

**Establishment of an expert panel in sensory
analysis and its involvement in cheese analysis**

Présenté par :

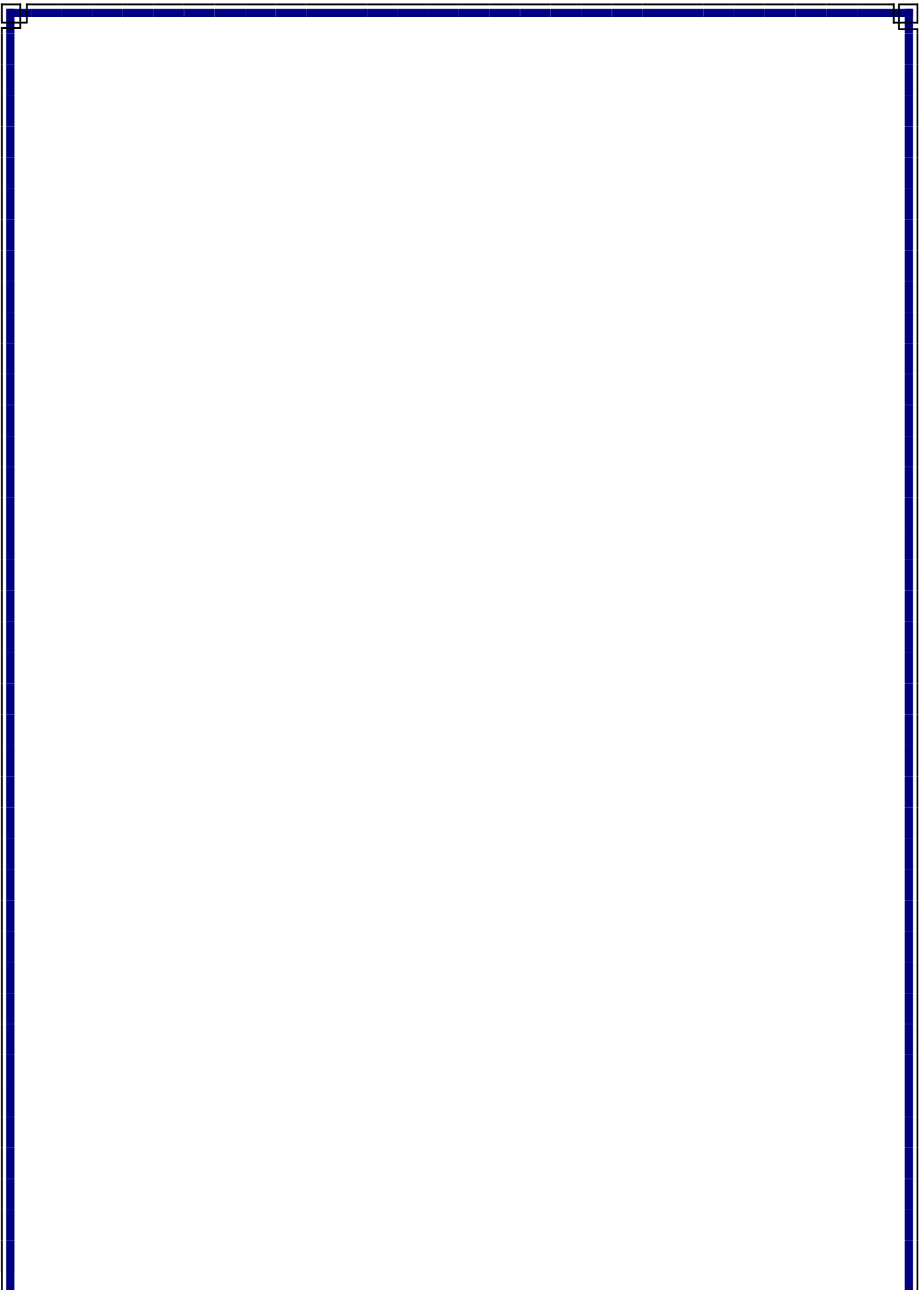
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♠ Yasmina & Yasmina ♠

Dedication

I dedicate this work

To my family, she who gave me a dignified education, her love made me
what I am today

In particular, to my father "*Hocine*" and my mother "*Zahia*" for the taste
for effort they have aroused in me, for their rigour.

To my brothers «*Makhlouf*» and «*Hamza*» my sisters «*Hamama, Soraya,
Fazia and Sabrina*» who have always supported and encouraged me during
these years of study

To my dear uncles, aunts, to my dear cousins and to all the members of
the family, young and old, may God always keep us united.

To those with whom I have found joy, to my dearest friend "*Yasmina*" and
"*Lydia*" in memory of our sincere and profound friendship and pleasant
moments, I dedicate this work to you and I wish you all happiness, and to
all my friends.



Yasmina ADNANE

Dedication

I dedicate this work

To my family, she who gave me a dignified education, her love made me
what I am today

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effort they have aroused in me, for their rigour.

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To my dear uncles, aunts, to my dear cousins and to all the members of
the family, young and old, may God always keep us united.

To those with whom I have found joy, to my dearest friend "*Yasmina*" in
memory of our sincere and profound friendship and pleasant moments, I
dedicate this work to you and I wish you all the best, and to all my friends.



Yassmina GHERBI

List of abbreviations

ISO: International Organization for Standardization.

IC50: the median inhibition concentration.

H%: moisture of the creams expressed as a percentage

N.A: Algerian standards

NS: *Nigella sativa L.*

ND: *Nigella damascena*

VRBL: Purple Crystal and Neutral Red Biliious Agar

PCA: Principal Completing Analyse

AHC: Agglomerative Hierarchical Clustering

PREFMAP: Preference MAPPING

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Sensory analysis is a science based on tasters called ‘panels’ who use the senses of smell, taste, touch, and hearing to measure sensory characteristics and degree of acceptability of the product. No material can reproduce or replace human sensory responses, making sensory evaluation as important as physico-chemical evaluation (**Depledge & Sauvageot, 2002**).

It is very important to follow a rigorous methodology to select a sensory analysis jury and to train it well in the particularities that may present the different properties of the products to be tested while familiarising the jury with the use of evaluation scales (**Lateur et al., 2001**).

With this in mind, our work aims to contribute to the renewal of the expert panel of generalists who are able to analyze different food products from the point of view of taste and smell (flavour). An expert panel was formed at the University of Bejaia a few years ago, but since many elements of this group have left the university and others are not necessarily available. So it is necessary to train other judges and to integrate them into the old panel. This panel will be used for the sensory analysis of various products developed as part of the end-of-cycle memoirs or as part of the research or simply for all the activities of the sensory analysis laboratory of the University of Bejaia in collaboration with other universities or with food production companies.

This work is divided into three main parts:

- Bibliographic summary on sensory assessment and the steps to follow for the establishment of a jury Expert in sensory analysis.
- Study material and description of the steps to follow to train the expert panel.
- Selection, training and validation of the trained expert jury.

It is with this in mind that we have opted for the production of a fresh cheese enriched with the seeds of black sativa and damascena, to bring to the cheese the beneficial properties of these plants as well as to improve its organoleptic characteristics essentially the aroma. Indeed, the genus *Nigella* L. includes about twenty species the Mediterranean regions has a reference to the intense black color is used in folk medicine as natural remedy number of diseases and conditions such as asthma, hypertension, diabetes, inflammation, bronchitis, headache, eczema, fever, dizziness and gastrointestinal disorders (**Margout, D et al., 2013**).

This study is divided into two parts:

- ❖ A bibliographic summary with general information on fresh cheese and nigella (*Nigella Sativa* L and *Nigella damascena*).
- ❖ An experimental study aimed first of all to make the determinations of total polyphenols and flavonoids and the study of the antioxidant activity and reductive power of the seeds of *Nigella sativa* L and *Nigella damascena*. In parallel, the preparation of five fresh cheeses A, B, C, D, E respectively (plain cheese, seasoned in *Nigella sativa* oil, seasoned in *Nigella sativa* powder, seasoned in *Nigella sativa* sheaths, seasoned in *Nigella damascena* sheaths). Finally, the study of the physicochemical, microbiological and sensory characteristics of these cheeses

Chapter I: Generalities on sensory evaluation and creation of generalist panel

I. Definition and purpose of sensory evaluation

According to the French norm NF ISO 5492, sensory analysis is defined as being “the examination of the organoleptic properties of a product by the organs of the senses” The human became the measuring instrument for sensory analysis methods to characterize and evaluate products using his five senses (sight, hearing, smell, taste and touch) (Castro et al., 2019).

The AFNOR norms define sensory evaluation as a "scientific method used to describe measure, analyze and interpret responses to products as perceived by the senses of sight, smell, touch, taste and hearing. Thus, the sensory analysis of a finished product or, upstream, of a material describes all of its so-called organoleptic properties. This comparative classification is carried out according to experimental protocol with the help of a tasters panel (AFNOR, 2004; Avramescu et al., 2014).

II. Sensory perception

It includes five types of sensitivities:

- **The visual sensitivity:** is the most known sensitivity and the most involved in the sensory evaluation, allowing the perception of shapes, colors and certain elements of texture (Sauvageot, 1998).

- **Taste sensitivity:** in humans, it is responsible for sweetness, saltiness, acidity and bitterness; it is mainly perceived at the level of the taste buds of the tongue (S Sauvageot, 1998).

- **Olfactory sensitivity:** is responsible for the perception of perfume (by the direct way) and aroma (by the retro-nasal way). When volatile substances are directly perceived through the nose, we speak about smell. On the other hand, the aroma is perceived

when the volatile substances are inhaled through the mouth (**Sauvageot, 1998, Sauvageot, 1998; Perrin, 2008**).

- **Auditory sensitivity:** is used in the evaluation of the texture of food products (crunchy, crispy...), specifically chips and wafers (**Sauvageot, 1998**).
- **Somesthetic sensitivity:** refers to the conscious sensations caused by the stimulation of body tissues that are not visual, auditory, gustatory or olfactory. They are caused by the excitation of various types of receptor nerve endings located in the skin covering and in more profound tissues such as the conjunctive or visceral tissue and the articular ligaments.

III. The different sensory thresholds

The different thresholds identified in the sensory analysis according to **AFNOR (2004) and Delwiche (2008)** are:

- ✓ **Perception or detection threshold:** the smallest value of a sensory stimulus required to awaken a perceived sensation.
- ✓ **Identification threshold:** the smallest value of a sensory stimulus required for recognition of the perceived sensation.
- ✓ **Differential threshold:** the smallest value of a sensory stimulus that causes a perceptible difference in sensation intensity.
- ✓ **The final threshold:** is the maximum value of a stimulus above which there is no discernible difference in sensation intensity.
- ✓ **The preferential threshold:** is the minimum quantitative value of a supra-preliminary stimulus, or the critical value of that stimulus, that corresponds to the appearance of an attraction or rejection response in relation to a neutral stimulus.

IV. Sensory tests

The test plays an important role in the various sensory assessment tests. As with any approach, the first step is to clearly define the problem and the constraints in order to choose the tests to be put in place knowingly. A sensory analysis can serve two purposes: it can study the differences between the proposed products or it can study their preferences. The

type of test will differ depending on the situation. We then have the choice between three types of events: Discriminative, descriptive and hedonic tests that aim to identify differences between products by analyzing and the relationships between them (**Anne-Sophie, 2008**).

IV.1. Discriminatory tests

Discriminatory methods are typically used when differences between the products to be compared are imperceptible. There are various techniques, but the principle remains the same: after evaluating the samples to be compared, participants must indicate whether the samples are identical or different depending on the variant used. The triangular test, the duo-trio test, and the A/Non-A test are three of the most common techniques (**Thomas, 2016**).

IV.2. Descriptive tests

Descriptive analysis can be used to determine the important sensory characteristics of a product. It allows also the classification of products in relation to each other on the basis of the component searched, or a global evaluation of the organoleptic characteristics of the products (**Anne-Sophie, 2008**).

IV.3. Hedonic tests

Hedonic methods focus on consumer preference and employ naive individuals, with no previous experience in sensory analysis. Evaluation tests aim to process the results of hedonic analysis of one or more products for comparison (**Thomas, 2016**).

V. Evaluation conditions

Sensory analysis requires special rooms, the same for all tests where distraction factors are reduced or minimal:

V.1. Local

It must allow for 04 types of activities:

- Administrative preparation of tests and their interpretation;
- Product preparation;
- Sensory evaluation of products;
- Organizing meetings with subjects (group work) (**Nicod, 1998**).

a) The Tasting Room

The tasting room must be quiet and comfortable with favorable atmospheric conditions and ambient lighting; the furniture, floors and walls must be neutral in color, odorless and easy to clean. The organization of the tasting room must avoid that the subjects influence each other (installation of fixed individual posts closed on three sides). The number of tasting posts is generally between 10 and 15 with comfortable chairs (Nicod, 1998).

b) The preparation room

This room is equipped according to the nature of the products evaluated; it must have all the material necessary for the preparation of the samples and their possible conservation as well as for the cleaning of the recipients (Nicod, 1998).

c) Presentation of samples

The samples must be representative of the product:

- **Size of the portions:** it is often limited by the amount of material available for the same small experience, the portion should represent the product;
- **Anonymity of samples:** The anonymous presentation is required when working on the qualitative and quantitative characteristics of products;
- **Coding of samples:** in order to identify the various anonymous products, each of them is given a code generally with three numbers;
- **Homogeneous presentation of samples:** all factors extrinsic to the product (temperature, quantity presented, container, etc.)
- **Method of presentation of samples:** in a general way we try to get as close as possible to the natural conditions of consumption;
- **Order of presentation of samples:** the influence of the order in which the subject tastes the products on his answers is avoided by changing this order from one subject to another and from one repetition to the other for the same subject (Stringler, 1998).

VI. Creation of a sensory analysis panel

A sensory analysis panel should be considered as a scientific instrument if it is possible to obtain reliable and valid results. The tests carried out with these panels must be

carried out under controlled conditions; the motivation of the group is the responsibility of the panel organizer.

VI.1. Definition of the sensory evaluation panel

A sensory evaluation panel is a group of person or subjects required to perform sensory evaluations. We can classify the groups of sensory evaluation according to their vocation: groups with a qualitative and quantitative vocation (expert subjects), and groups with a hedonic vocation, that is to say naive consumers (Nicod, 1998).

VI.2. Different types of subjects

Different types of subjects can be distinguished according to the following figure as follows:

- The naive subject: the person who does not meet any particular criteria;
- The initiated subject: the person who has already participated in a sensory trial;
- The qualified Subject: The Subject Selected for Sensory Testing;
- The expert subject: the person who, through knowledge and experience, is competent to provide advice in the areas on which he is consulted (ISO8586-2, 1994).

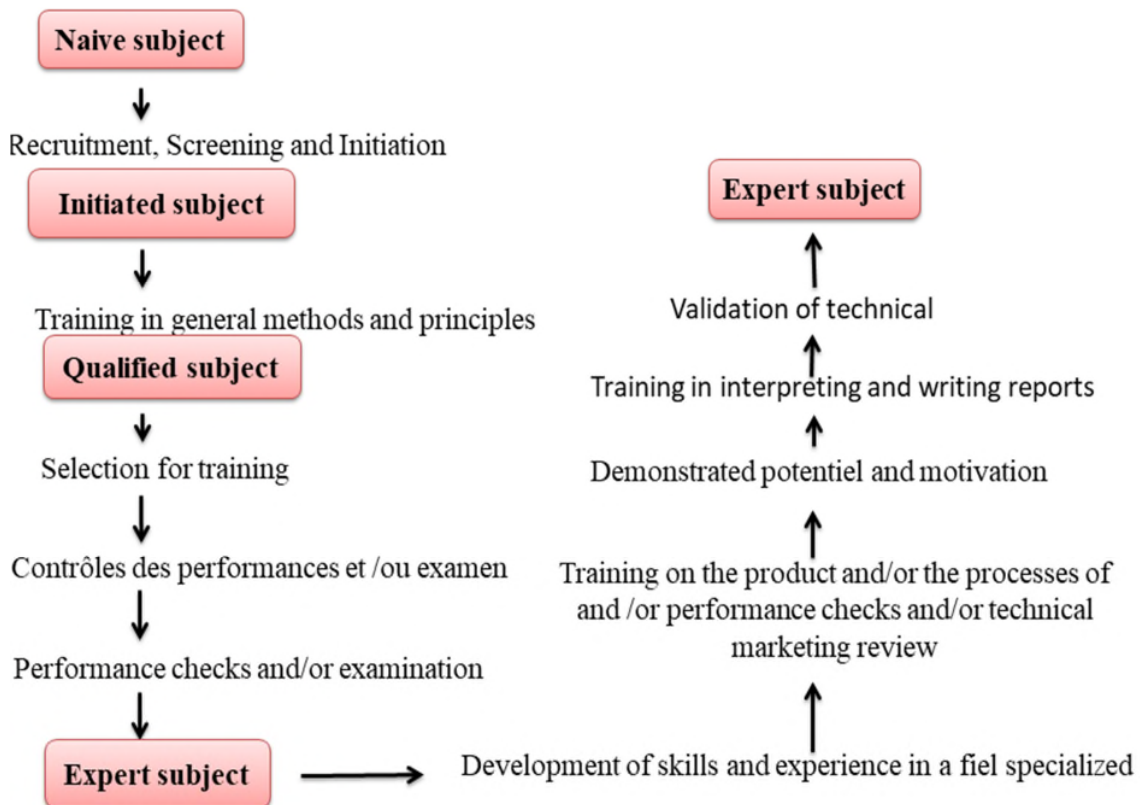


Figure 01: Different types of tasters (ISO 8586-2, 1994).

VI.3. Criteria for the selection of expert subjects

According to **Perrin (2008)**, the subjects to be trained must satisfy different criteria:

- **Trueness:** the agreement between several measurements

The trueness of a measurement is the closeness of agreement between the result of the measurement and the true value of the quantity being measured.

- **Fidelity:** repeatability and reproducibility

Fidelity is the ability to give, under defined conditions of use, very similar responses when applying the same input signal.

- **Accuracy:** agreement between subjects

Accuracy is a combination of correctness and precision of measurement.

- **Sensitivity:** the discriminating power

The final criterion for assessing the reliability of a method is its sensitivity as well as the ability of the method to differentiate the products being evaluated (**Perrin, 2008**).

VI.4. Creation of the sensory evaluation panel

The verification instrument of the sensory analysis is the panel of individuals who have been recruited and trained to perform specific sensory evaluation tasks. Their recruitment, training, performance monitoring, instruction and motivation are the responsibility of the panel manager. It is essential for the good functioning of the panel that "organizers" are effectively involved in the preparation and management of the panel (**Watts et al., 1989**).

The formation of a sensory evaluation group involves the following steps:

- Recruitment and preliminary screening;
- In-depth selection;
- General and specific training;
- Control (reliability of responses, repeatability, etc.) (**Nicod, 1998**).

VI.4.1. Taster Recruitment

The panels of expert tasters, as well as those made with amateur tasters, can usually be found among the staff of the institution or organization conducting the research. In general, they will be interested in participating if they feel their input is important (**Watts et al., 1989**). The types of recruitment can be distinguished:

- **Recruitment of the internal group:** it is important that there is a general intention to Promote sensory evaluation in the company and especially in the direction.
- **Recruitment of the external group:** it requires the intervention of media, newspaper Article, classifieds, telephone, etc. (**Nicod, 1998**).

In order to help recruit them, all tasters should be asked to complete questionnaires (see appendix) that will make them specify:

- their taste for food;
- their level of interest in the project;
- the constraints to which they are subjected in terms of food or the allergies they may have and to specify the times when they would be free for the panels;

It is also necessary to keep general information on each participant in a sensory analysis panel (**Watts et al., 1989**). It is useful to have, for each subject, an information sheet containing names, address and telephone number, and time availability (**Nicod, 1998**).

Instructions for tasters

- Potential tasters should be invited to attend the panel in groups of no more than 10 people;
- The trainer can explain the importance of sensory testing, show them the facilities and answer any questions they may have;
- Those who participate only in on-site panels to assess the acceptability of a product (panels of amateur tasters) do not need further training;
- Explaining the analytical method and procedures used will reduce the confusion and will make it easier for the tasters;
- It is important that they have a good understanding of the procedures used and how to complete the questionnaire in order to participate in the tests on the same basis;
- Before participating in a panel, avoiding eating, drinking or smoking for at least 30 minutes before testing (**Watts et al., 1989**).

VI.4.2. Selection of persons for the expert panels

Individuals who agree to participate in expert tasting panels must be tested to determine if they have good sensory acuity. So they are asked to identify basic flavors and current smells (Watts et al., 1989).

It is important that these people have been able to taste, smell, touch the solutions or products used later in the selection tests, knowing their names (verify that the subjects are not agueusic or anosmic to the different products to be evaluated), by carrying out the following tests:

- **Pairing test:** these tests determine the ability of subjects to recognize substances among others.
- **Discrimination tests:** these tests determine the ability of subjects to detect differences between products.
- **Descriptive ability tests:** these tests evaluate the level of verbal creativity of individuals, their ability to explain the terms they propose with known and explicit references.
- **Identification tests:** they should only be used to check the subjects' ability to memorize.

VI.4.3. Selection Criteria

To constitute an effective descriptive sensory analysis jury, a selection must be made to the following criteria:

- **Health:** Tasters must be in good health and should withdraw if their health Condition is likely to interfere with the normal functioning of taste and smell like: colds, allergies, medicines and pregnancy.

- Do not smoke, chew gum, eat or drink at least 30 minutes before the test begins (Poste et al., 1991).
- **Motivation:** by asking the subject to describe in some line the reasons that lead him to participate in sensory evaluation (it is important that the subject be motivated).
- Age and sex are not selection factors. Women's performance is certainly on average better than that of men (at any age) (Nicod, 1998).
- **Availability:** The availability of tasters during training and analysis is essential. People who have to travel frequently and some production employees cannot sit on a panel.

- **Punctuality:** Punctuality is essential, not only to avoid wasting the time of colleagues, but also to maintain the integrity of the experimental and sampling plan.
- **Verbal Communication:** Depending on the evaluation method used, tasters must demonstrate a higher ability to communicate descriptive evaluations that define and describe the different characteristics of the products (**Poste et al., 1991**).

VI.4.4. Taster Training

Training should be designed to help tasters make valid and reliable judgements independent of their personal preferences (**Watts et al., 1989**).

Training is an important phase in the formation of a group. According to (**Nicod,1998**) it must allow the subjects to:

- ✓ Become familiar with the vocabulary specific to sensory analysis;
- ✓ Memorize textures, flavors, smells and aromas characteristic;
- ✓ Renew these elements in a complex product if it has very marked characteristics;
- ✓ Calibrate over a range of known concentrations to evaluate intensities.

VI.4.5. Performance control of tasters

The performance of the tasters must be monitored during the training period to determine the progress made. This allows training to be tailored to samples and sample characteristics that pose difficulties in identifying and evaluating tasters.

It is also possible to check the performance of the tasters during the sensory study by comparing repeated evaluations. This ensures that tasters have reliable, consistent performance and will indicate when they will need additional training or more motivation (**Watts et al., 1989**).

Chapter II: Generalities about cheeses

This study consists in the elaboration and validation of a panel of general experts in sensory analysis and its implication in the analysis of cheeses. Of which it is necessary to give a small outline on the cheeses as well as on the fresh cheeses which are used as support to the sensory evaluation in the practical part.

I. Definition of cheese

The name cheese is reserved for fermented or unfermented dairy products, ripened or not obtained from the following exclusively dairy materials of origin: milk, partly or totally skimmed milk, cream, fat, buttermilk, used alone or in a mixture and coagulated in whole or in partial elimination of the aqueous part (Joffin and Joffin, 1990).

II. Cheeses Classification

Cheeses are difficult to classify because of their variety: cooked or raw, fresh, hard or soft, pressed, bloomy or washed rind, etc. This variety is further complicated by the overlapping characteristics that make up the classification (Mietton, 2014).

Lenoir et al. (1983); Almena-Aliste and Mietton (2014) give a synthetic and instructive vision of the diversity of cheese making. The methods of coagulation, draining and maturation of the curd lead to a great variety of cheeses as illustrated in Figure 02.

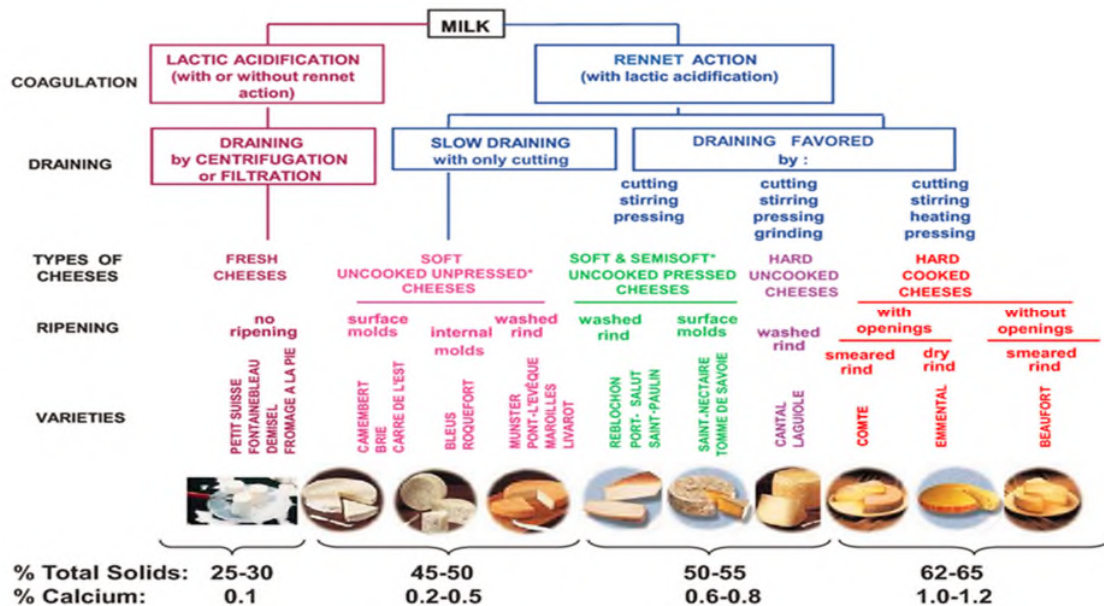


Figure 02 : Classification of cheeses (Almena-Aliste and Mietton, 2014)

III. Composition and nutritional value

Cheese is particularly rich in protein, water, bioactive peptides, amino acids, lipids, fatty acids, vitamins and minerals as shown in Table I (Walther et al., 2008).

Table I: Overall composition of some cheeses (Walther et al., 2008).

| Constituents | Fresh Cheese | Soft Cheese | Pressed Cheese |
|-----------------------------|--------------|-------------|----------------|
| Moisture (g/100g of cheese) | 80 | 50 | 48 |
| Carbohydrates (g/100g) | 4 | 4 | 2,5 |
| Fat (%) | 7,5 | 24 | 22 |
| Protein (%) | 8,5 | 20 | 18 |
| Calcium (mg/100g) | 100 | 400 | 680 |
| Sodium (mg/100g) | 40 | 700 | 1650 |
| Vitamin A (IU) | 170 | 1010 | 1200 |

IV. Definition of fresh cheese

Fresh cheese is a cheese that is ready for consumption a few hours after it is made, it means it is not ripened. It has a very short time limit of consumption because of its high water content, it lasts from one to seven days or 12 months if it is marinated in brine or in oil. It is very easy to distinguish due to its white color, shine and lack of rind (Harbutt et al., 2009).

V. Fresh cheese analysis

The physico-chemical analyses that are generally carried out on fresh cheese are: the analysis of pH, titratable acidity, fat, proteins, carbohydrates, ashes, mainly calcium, moisture and dry matter (Hamdy et al., 2021)

Fresh cheese is a favourable product for the development of micro-organisms belonging to very diverse groups or species, which can come from several sources: milk, leavening, salt or brine, equipment of the cheese factory or atmosphere of the locals . The micro-organisms present in the cheese can be classified in 3 categories (Hermier et al., 1992; Hadjilouka et al., 2020):

- **Useful microorganisms** like lactic acid bacteria;
- **Microorganisms responsible for alteration** like coliforms;
- **Potentially pathogenic microorganisms** such as listeria

Chapter III: Generalities about *Nigella*.

I. *Nigella sativa* L. (black cumin)

Nigella sativa L. (NS) is an annual herbaceous plant belonging to the *Ranunculaceae* family (figure 03). It is widely grown in the Mediterranean countries, Middle East, Eastern Europe and Western Asia. The black cumin seeds taste hot-peppery and have been used as a spice in several foods, such as bread, yogurt, pickles, sauces and salads, but also in the preparation of black cumin paste, being considered as a valuable functional food (Toma et al., 2015; Sharma et al., 2019).

They are extensively used in traditional medicines in Pakistan, India, China, Saudi Arabia and the countries bordering the Mediterranean region for the treatment of asthma, cough, bronchitis, headache, rheumatism, fever, kidney and liver disorders, influenza, eczema, and as a diuretic, lactagogue, carminative and vermifuge (Rajsekhar et al., 2011; Toma et al., 2015; Salehi et al., 2021). Recent scientific investigations on *Nigella sativa* L. seeds and its oil indicate a number of bioactivities for the plant, which include anticarcinogenetic, antiulcer, antibacterial and antifungal, antihypertensive, hepatoprotective, anti-inflammatory, antipyretic and analgesic, as well as antioxidant activities such as quenching reactive oxygen species, prevention of rheumatoid arthritis in rat models, and antihyperlipidemic (Ahmad et al., 2013; Toma et al., 2015; Sharma et al., 2019).

Black cumin produces a wealth of phytochemicals including fixed and volatile oils, proteins, flavonoids, glycosides, alkaloids and saponins (Telci et al., 2014; Toma et al., 2015; Salehi et al., 2021).



Figure 03: Different parts of the plant *Nigella sativa* L. (Rajsekhar et al., 2011).

II. *Nigella damascena* L. (lady-in-a-mist)

Nigella damascena L. (ND), commonly known as lady-in-a-mist or ragged lady, is an annual plant belonging to the buttercup family *Ranunculaceae* and widespread throughout temperate regions of Europe (figure 04). ND is grown as an ornamental plant. Its seeds are used in traditional medicine because of their analgesic, anti-edematous and antipyretic effects and, due to their sweet scent of strawberry, to prepare food (Toma et al., 2015; Badalamenti et al., 2022).

The two *Nigella* species have been used since ancient times for medicinal purposes but also as a food or preservative for foods. Due to its adaptability, NS had a higher commercial value and a larger growing area than ND *has* had, and as a consequence there has also been more investigation into the chemical composition and biological activity of NS *than* that of ND. (Telci et al., 2014, Toma et al., 2015; Badalamenti et al., 2022).

Nigella damascena L. contain fatty and essential oils, proteins, alkaloids, phenolic compounds, flavonoids and saponins (Fico et al., 2000;Toma et al., 2015; Badalamenti et al., 2022).



Figure 04: Different parts of the plant *Nigella damascena* L.

Experimental part

I. Materials and methods

The first group of experts for the analysis of the flavour of food products was formed at the University of Bejaia in 2009; it was composed of 13 judges. A second group of specialized experts composed of 17 judges, was formed in 2016 for the sensory analysis of cheese. But after several years, many elements have left the University of Bejaia and others lack availability. Currently, only 10 of them come regularly to perform analysis in the laboratory of sensory analysis. Due to the high number of analyses that are carried out in the laboratory, especially during the period of completion thesis. It is necessary to train new subjects who will progressively integrate the old group, which is the objective of this study.

All the tests were carried out at the sensory analysis laboratory of the Department of Food Sciences (University A.MIRA, Bejaia).

A group of people of different age groups: teachers, laboratory technicians, engineers and faculty staff are invited to participate in the selection of the group.

I.1. Procedures for setting up the expert panel

To constitute a sensory evaluation group, specific procedures are used, in particular: the **Spencer procedure (1971)** for the creation of a panel (general experts), for food flavor analysis.

During a period of two and a half months, potential jury members were subjected to tests based on taste and smell, according to a standardized method (AFNOR) which is developed in order to train a group of generalists who, after training, would be able to evaluate different foodstuffs from the point of view of flavor (odor, aroma, and taste): this involves the olfactogustive complex and common chemical sensitivity (**Sauvageot, 1998**).

Spencer procedure has 3 phases of selection:

- 1- Pre-selection;
- 2- Selection;
- 3- Training.

At the end of each test, the subject is declared able or not able to continue to the next step (Sauvageot, 1998).

I.1.1. Pre-selection step

In this phase, we informed the teachers, technicians, engineers and staff of the Faculty of Natural Sciences and Life and even other faculties of the University of Bejaia; that sensory

analysis tests of pre-selection would take place, during the period of May to June 2022. For this purpose, 50 volunteers were presented, a questionnaire were distributed (Annex I) in order to collect information on their sensory acuity, their availability and their motivation on the subject.

I.1.2. Selection step

This selection phase is based on the Spencer procedure, following three steps:

- Step One: Pairing test
- Stage Two: Discrimination Trials (Differential Test)
- Step 3: Test to assess the ability of subjects to identify or describe an odour.

Before each evaluation, the tasting room must be thoroughly cleaned and prepared with the necessary equipment for the various tests to be carried out: tasting booths (Figure 05), spittoon, questionnaire, etc.



Figure 05: Photograph of the tasting posts (stalls)

Step 1: Identify the four principal flavors

The purpose of this step is to detect the incapacities of the subjects to distinguish the four fundamental flavors (sweet, salty, bitter and acid). This step consists of presenting in a random order four sapid solutions: sucrose, sodium chloride, caffeine and citric acid, to identify the nature of each, the subject is informed that the sample can be: sweet, salty, bitter or acid (Sauvageot, 1998). The solutions prepared are presented in the following table:

Table II: Concentration of the four fundamental flavors

| Solutions | Concentration (mol/l) |
|-----------------|-----------------------|
| Salt solution | 0,034 (mol/l) |
| Acid solution | 0,010 (mol/l) |
| Bitter solution | 0,005 (mol/l) |
| Sweet solution | 0,058 (mol/l) |

04 cups coded 1, 2, 3 and 4 were prepared, each containing one of the 4 flavors. The subject is asked to identify the four basic flavors according to the following questionnaire:

| | | | |
|--|--|--------------|--|
| <p>Training for the selection of expert judges in food sensory analysis Test 01: Identifying the Four Fundamental Flavors</p> | | | |
| <p>Last and First Name: Date: Post No.:</p> | | | |
| <p>In order to form a jury expert in sensory analysis of food, the first test corresponds to the ability to distinguish the 4 fundamental flavors: acid, bitter, sweet and salty.</p> | | | |
| <p>Four cups coded 1, 2, 3 and 4 are presented to you, you are asked to taste the samples successively without swallowing and identifying the four fundamental flavors. Each flavour must be marked with the corresponding cup number.</p> | | | |
| <p>N.B: Please rinse the mouth after each sample.</p> | | | |
| acid | | Salt | |
| Bitter | | Sweet | |
| Observation | | | |
| <p><i>Thank you for your participation.</i></p> | | | |

Figure 06: Evaluation questionnaire for Test 1.

Step 2: The ability to distinguish sensory thresholds from the four fundamental flavors.

The purpose of this step is to determine sensory acuity. The subject is asked to classify six concentrations, in order of increasing intensity prepared from the four stock solutions: sweet, salty, bitter and acid, no error is also tolerated. From each solution previously prepared, a series of arithmetic dilutions has been prepared in accordance with (AFNOR, 1999), as indicated in the **appendix N°II**.

06 coded cups were prepared of increasing concentration for each basic flavor; the subject is asked to identify the flavor whose concentration increases each time he goes from one dilution to another, according to the following questionnaire:

Training for the selection of expert judges in food sensory analysis
Test 02: Differential classification of the four fundamental flavors

Last and First Name:**Date:** **Post No:**

06 cups coded 1, 2, 3, 4, 5 and 6 are presented to you, containing the dilutions of a single flavour and in a ascending order respectively. You are asked to taste the samples successively without swallowing and identify the flavor with the increase in concentration each time you switch from one dilution to another. Responses will be provided in the newsletter as follows:
 The answers will be given in the bulletin as follows:
0: for no sensation.
X: for appearance of a sensation.
XX: for identification of the solution
XXX: for the first feeling of difference in concentration (a cross is added to each increase in sensation)
 Please circle the flavor identified in each dilution series and rinse the mouth after each sample.

| Sample | 1 | 2 | 3 | 4 | 5 | 6 | Identified flavor |
|---------------|----------|----------|----------|----------|----------|----------|--------------------------------------|
| Answer | | | | | | | Salty, Sweet Bitter, acid |

Thank you for your participation

Figure 07: Differential test Evaluation questionnaire for test 2.

Step 3: Odour Recognition

The purpose of this test is to assess the potential of subjects to describe and communicate information about sensory responses and to become familiar with certain smells.

The subject must smell the 20 substances presented in annex III, the subject is invited to identify them or at least describe them. Subjects have 45 seconds per substance (**Sauvageot; 1998**).

| Training for the selection of expert judges in food sensory analysis | | | | | |
|--|---------|-------------------|---------|-------------------|---------|
| Test 03: Odour Recognition | | | | | |
| Last and First Name: | | | | | |
| Date: | | | | | |
| Post N°: | | | | | |
| The subject must smell the 20 substances presented in the table below, memorize them and then identify them in the 20 tubes presented. The N° of the corresponding tube must be written opposite each odorous substance: | | | | | |
| Pure body | | Essential oils | | Aromas | |
| Odorous substance | Tube N° | Odorous substance | Tube N° | Odorous substance | Tube N° |
| Ammoniac | | Lavender | | Cherry | |
| Acetic acid | | Thyme | | Vanilla | |
| Benzaldehyde | | Eucalyptus | | Strawberry | |
| Phenol | | Rosemary | | Toffee | |
| Butyric acid | | Lentistic | | Honey | |
| | | Lemon | | Pistachio | |
| | | Cinnamon | | Banana | |
| | | Clove | | | |
| <i>Thank you for your participation.</i> | | | | | |

Figure 08: Odour Recognition Questionnaire for test 3

After having validated the 2 phases of the selections, the judges who had given incorrect answers were eliminated.

I.1.3. Training step

This phase consists in organizing training sessions on the analysis of cheese from the point of view of taste and aroma. For this reason we proceeded to the preparation of fresh cheese, after which it was analyzed by the panel that was trained.

The training procedure of the judges aims to:

- Learn how to evaluate cheeses;
- To give purely qualitative judgments without taking into account preferences;
- Compare their perception with that of other judges in order to reduce inter-individual differences;
- Increase their sensory knowledge (**Gallerani et al ., 2000**).

We used fresh cheese as an evaluation support in the sensory analysis of cheese, since it is a neutral cheese, without specific taste, smell or texture.

I.2. Preparation and analysis of fresh cheese

I.2.1. Preparation of fresh cheese

We used fresh cheese enriched or not with *Nigella* seeds as an evaluation support in the sensory analysis of cheese, since it is a neutral cheese, without taste, smell or specific texture.

The main materials used for the preparation of cheese are:

- Raw cow's milk was purchased from a farm in the village of Feraoune (Bejaia);
- Milk powder from a local supermarket;
- The rennet powder and lactic ferments from the laboratory of sensory analysis
- Seeds of 2 species of *Nigella* (*Nigella sativa* L. and *Nigella damascena* L.) and black cumin oil for the enrichment of fresh cheese.

- **Reception and filtration of milk**

As soon as the milk arrives, it is filtered to remove physical pollutants and its pH is measured to ensure its freshness.

- **Mixture preparation and heat treatment of milk**

Before beginning the manufacturing, a liter of milk or a mixture must be heated. The harmful bacteria present in vegetative form are then eliminated by the heat treatment (at 90°C), reducing the overall flora (**Carole and Vignola, 2002; Luquet, 1994**)

- **Addition of rennet and ferments**

After cooling the milk about 30-34°C, 2 mL of liquid rennet have been added followed by good stirring. A firm curd is obtained after 45 min of rest. Coagulation corresponds to a physico-chemical change of casein micelles under the action of proteolytic enzymes (rennet). These lead to the formation of a network three-dimensional protein called coagulum or gel, which then allows the expulsion of a large amount of water and soluble materials (**Eck and Gillis 2006**).

- **Filtration and draining**

The curd is recovered after filtration using a sieve and a fine cloth. The draining of the curd is done manually by pressing lightly; it allows the progressive elimination of the remaining whey. It is necessary to exert a mechanical action (centrifugation or filtration) to obtain a sufficiently drained cheese (**Roux and Rueff, 2006**)

I.2.2. Preparation of extracts

In order to assess the phenolic compounds and antioxidant capacity of the made cheeses, as well as the influence of enrichment with the two species of black cumin on the phenolic compound content and antioxidant activity of the cheeses. Consequently, two extractions were performed on the five developed cheeses as well as on the seeds of *Nigella sativa* L. and *Nigella damascena* L.

I.2.2.1 Preparation of the cheese extract

The **Apostolidis et al., (2007)** procedure was modified slightly to prepare the cheese extract; 1g of cheese samples and 10 ml of distilled water were mixed for 2 minutes. After centrifuging homogenized samples twice for 10 minutes at 4000 rpm, the supernatant was collected. Each sample was kept at 4 °C.

I.2.2.2 Preparation of *Nigella* extracts

Nigella sativa L. and *damascena* L. seeds served for the enrichment of fresh cheese. The first species was purchased from herbalist in Bejaia. And the second was purchased from a market in France. The dried seeds were ground using an electric grinder, then sieved through a granulometric (500µm) in order to recover the finest powder (**Figure 09**).

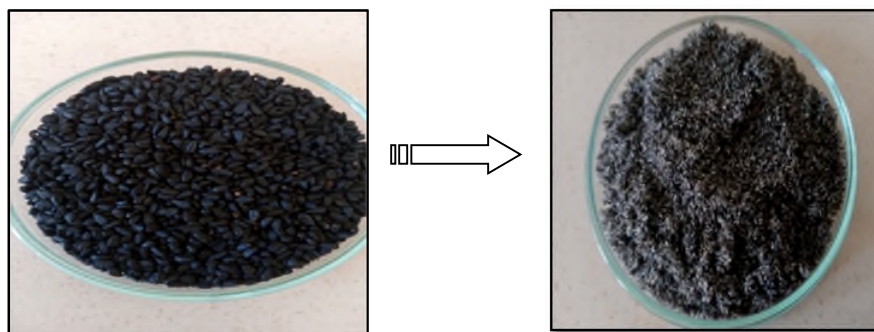


Figure 09: Photography of the preparation of *Nigella* seed powder.

In this study, the extraction was carried out by combination of tow method described by (**Venkataswamy et al., 2017; Yacine et al., 2013**) slightly modified. It entails macerating the fine powder obtained with a ratio of 1:10 (m/v) in the mixture of methanol/water (80:20, v/v) for 30 minutes while being continuously stirred and shielded from light with a magnetic blender. After that, the solution was centrifuged twice for 10 minutes at 4000 rpm. After being separated; the supernatant was continuously stirred into a mixture of water and

methanol that was twice as large. The precipitate went through a filtering and methanol washing process before being dried at 40°C until stabilization.

This is how the extracted yield is determined:

$$\text{Extraction yield} = \frac{\text{dry matter weight}}{\text{initial powder weight}} \times 100$$

I.2.3 Quantification of phenolic compounds in cheese and *Nigella* extracts

I.2.3. 1 Total phenolic content

The total phenolic was assayed (**Figure 10**) according to the method of by using the Folin-Ciocalteu reagent (Vazequez et al., 2015).

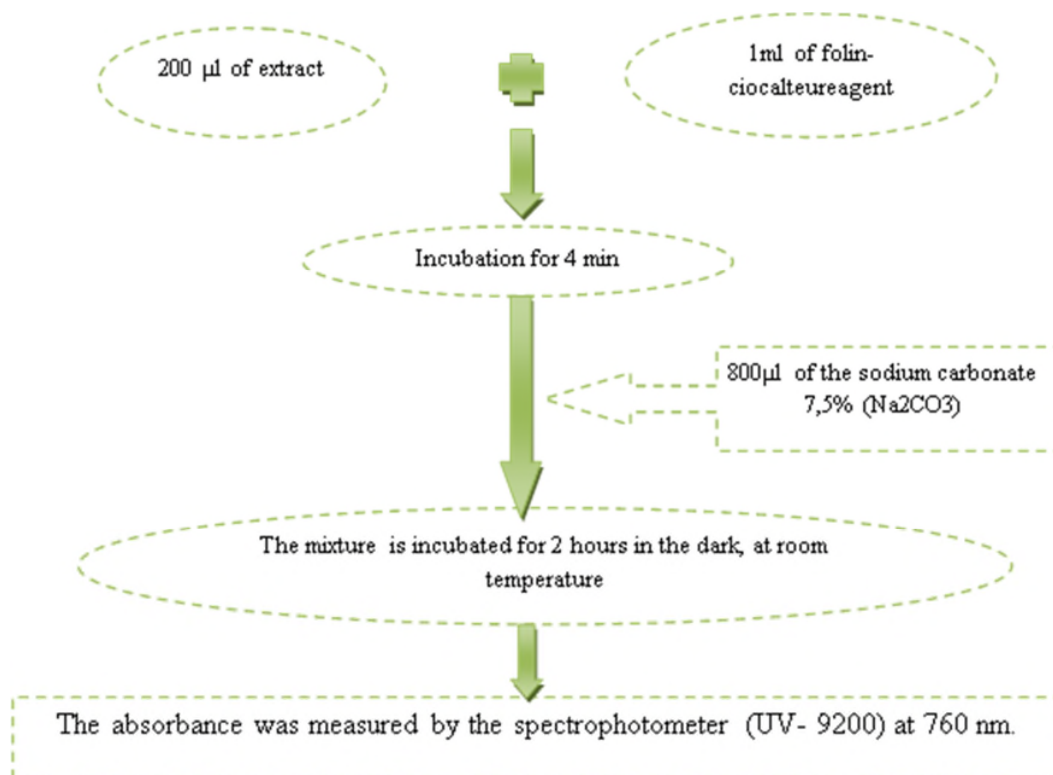


Figure 10: Phenolic content protocol by Vazequez et al. (2015)

All samples were prepared at room temperature and in low-light conditions. The intensity of the blue colours shows the presence of phenolic content (Bucic et al., 2007).

The Determination of total phenolic content was carried out and calculated from the calibration curve obtained with Gallic acid, which was used as a standard and results were expressed in

mg of Gallic acid equivalent per gram of dry extract (**mg AGE/g dry extract**) or (**mg AGE/100g of cheese**).

I.2.3. 2 Flavonoids content

The flavonoids in our extracts are measured using the aluminum chloride technique (Figure 11), the yellow color indicates that flavonoids are present in the extract (**Katz et al., 2011**).

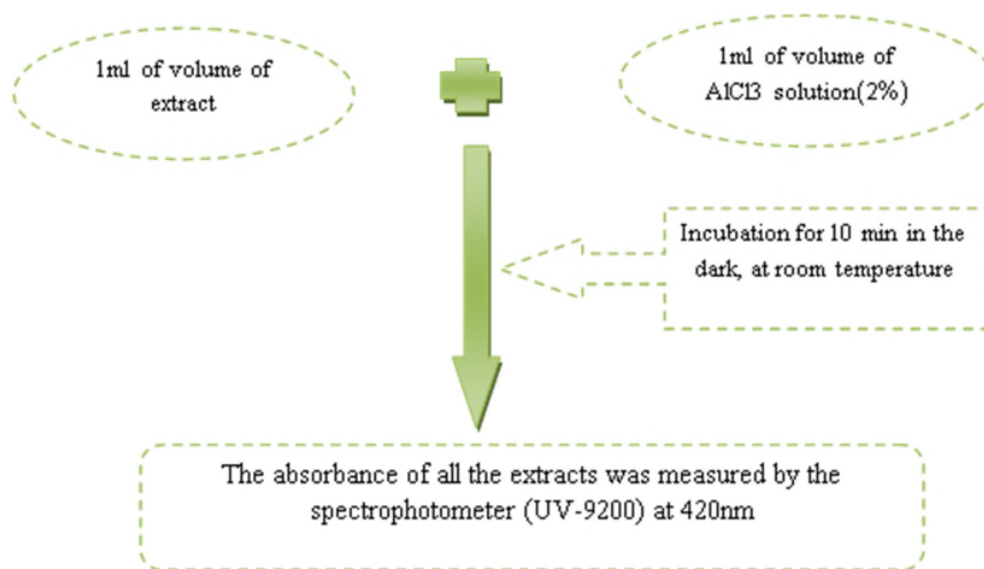


Figure 11: Flavonoids content Protocol (**Katz et al., 2011**).

The Determination of flavonoids was carried out in a duplicate and calculated from the calibration curve obtained with Quercetin, the results were expressed in mg of quercetin equivalent per gram of extract (**mg QE / g dry extract**) or (**mg AQE/ 100g of cheese**).

I.2.4 Antioxidant activity of cheese and *Nigella* seeds

I.2.4.1. 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH•) inhibition assay

The DPPH• radical scavenging assay has been used constantly to evaluate the ability of antioxidant molecules to scavenge free radicals, and it is considered one of the straightforward and common colorimetric approaches to examine the antioxidant properties of pure and natural compounds (**Mishra et al., 2012**).

The reduced form of DPPH is produced when the solution of DPPH° is combined with a molecule that may donate an electron or a hydrogen atom. This results in the loss of the purple

color and the appearance of a residual pale yellow color because of the presence of the picryl group (**Figure 12**). The maximal absorbance of the DPPH radical lies between 517 and 520 nm, and it declines as the radical's concentration is lowered (**Marxen et al., 2007**). The intensity of the purple color decreases after being reduced by an electron or a hydrogen radical given by an antioxidant molecule (**Alsaraf et al., 2020**).

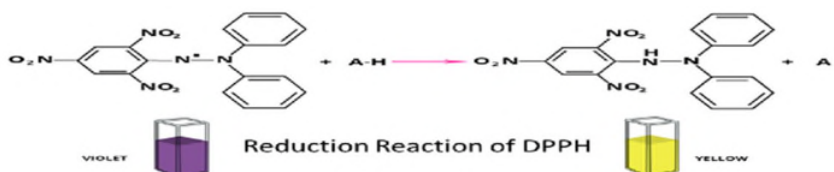


Figure 12: Reducing form of DPPH (**Khan et al., 2021**).

The protocol used is presented in the following figure:

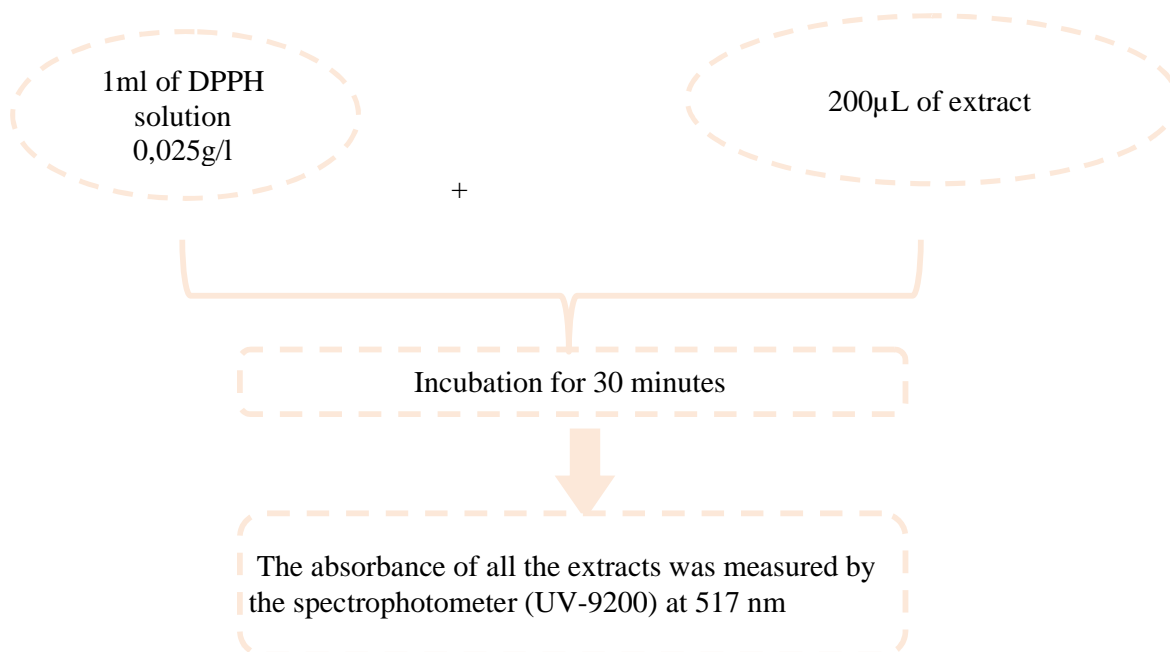


Figure 13: DPPH protocol (**Apostoldis et al., 2007**).

The experiment was performed in triplicate. The % inhibition of DPPH radical was computed by the following formula:

$$\% \text{ Radical scavenging activity} = \frac{Actl - A_{sam}}{Actl} \times 100$$

Where:

A ctl = absorbance of the control;

A sam = absorbance of the sample extract;

I.2.4.2 Iron reducing power test

Reducing power depends upon the capacity of electron transfer and may therefore, act as a prominent indicator for its antioxidant potential (Koruthu et al, 2011). In this study, the yellow color of the test solution changes to green depending on the reducing of test sample. The presence of reducers in the solution causes the reduction of the F^{3+} / ferricyanide complex to the ferrous form. The intensity of the green color was measured at 700nm (Gulcin et al, 2005).

The protocol is presented in the following figure:

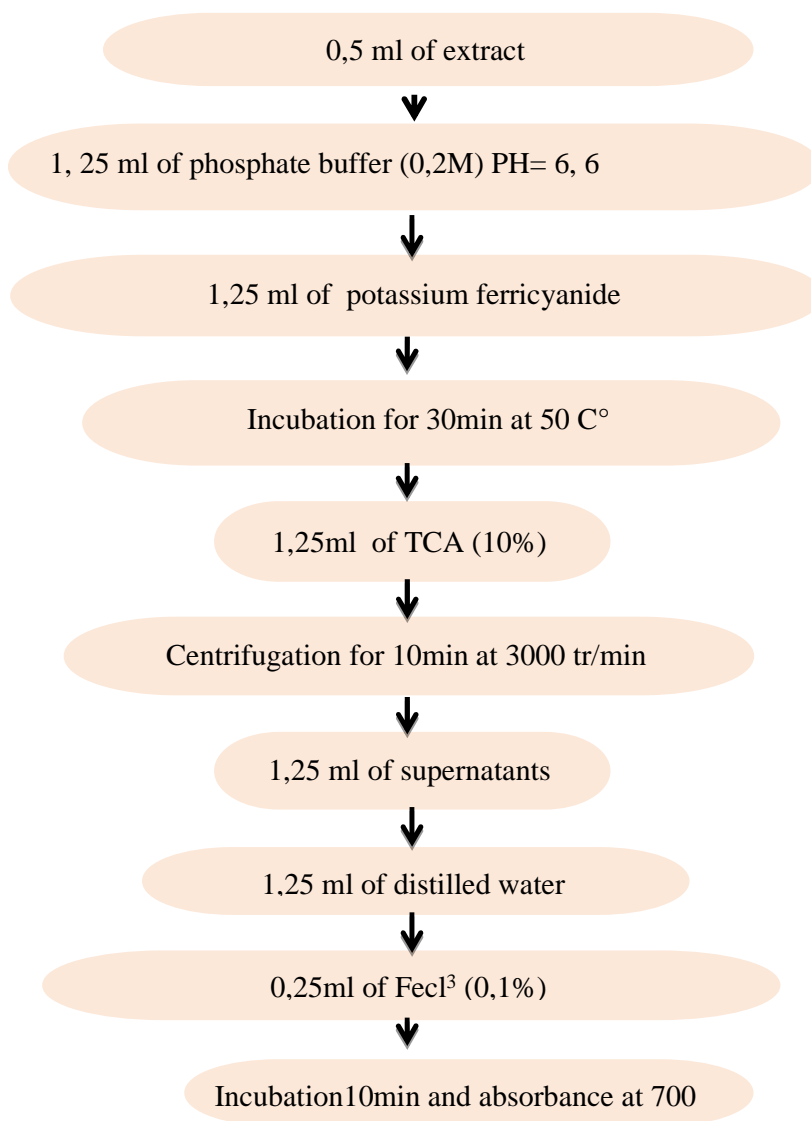


Figure 14: Iron reducing power test

1.2.5. Physico-chemical analysis of cheese

The physico-chemical analysis of cheese was conducted at laboratory of physico-chemistry of food at the University of Bejaia and at a private laboratory of food quality control (Idres laboratory).

1) pH measurement

The pH depends on the concentration of protons in a medium; it is the logarithm of the molar concentration of the hydronium ion (H_3O^+) (Jaques, 1998). We can determine a food's freshness by measuring its PH (Carol and Vignola, 2002).

1g of cheese is mixed with 9 ml of distilled water and homogenized. The pH of the sample is determined after one hour using a digital pH meter where the electrode was inserted directly into the sample. This procedure was performed in triplicate (Owusu-Kwarteng et al., 2012).

2) Titrable acidity

The titratable acidity expressed in degree Dornic ($^{\circ}D$), or in gram of lactic acid in 100g of food product was determined after titration with a solution of NaOH (N/9) in the presence of the colored indicator phenolphthalein at 1% (w/v) considering that 1 ml of NaOH solution corresponds to 0.01 g of lactic acid per cent or $10^{\circ}D$ (Shori et Baba, 2013).

10g of cheese were added to 90ml of distilled water. The mixture is well homogenized, then 10 ml of this suspension is titrated by the soda N/9, in the presence of phenolphthalein at 1% (w/v).

The phenolphthalein indicates the limit of neutralization by color change (pale pink), the results are expressed in degree Dornic by gram of cheese ($^{\circ}D/g$) (Zoinoldin et Baba, 2009).

3) Evaluation of moisture content

The moisture of a food is the amount of free water it contains. Its determination is done by drying in a ventilated oven at $105^{\circ}C$ until the mass of the food remains constant (AFNOR, 1999).

3 Petri dishes containing (2g) of each cheese sample were placed in a ventilated oven at $105^{\circ}C$ for 3h.

$$H (\%) = \frac{(W-W')}{W} \times 100$$

W: the initial weight of sample (g)

W': the loss in weight (g) on draying

4) Carbohydrate determination

The BERTRAND method is applied for the determination of carbohydrates in cheese samples. This method allows the determination of oses and reducing osides. It is a reductimetric method based on the reducing properties of oses towards the ions Cu^{2+} ions of Fehling's liquor in basic medium and at boiling.

- **Procedure**

Weigh 5g of cheese in an erlenmeyer flask (PE test tube), Add 10ml of distilled water and shake to dissolve the cheese, Add 2ml K^+ 15% ferrocyanide and 2 ml zinc acetate 30% (defecation) and let 15min, Add 10ml of HCL 83g :L to hydrolyze water bath Neutralize the HCL with Na_2CO_3 and adjust to 100ml with distilled water then filter, Put 20ml of Fehling's liqueur A and 20ml of Fehling's liqueur B in an Erlenmeyer eyer flasflask and add 20 ml of the filtred solution (Test portion PE ').

Heat to boiling for 3 min and allow cooling by tilting the Erlenmeyer flask, Filter and washing the precipitate to remove any excess Fehling's liqueur with hot distilled water.

The expression of the results is as follows: calculation of (X) according to the formula below:

$$(Cb \cdot f) = X$$

Cb: burette drop

f : Correction factor

Find the equivalent of (X) on the BERTRAND table: it corresponds to (Y) which is replaced

In the formula below:

5) Determination of fat

After dissolving the cheese proteins with sulfuric acid, the fat is separated by centrifugation in a butyrometer, the separation being favored by the addition of a small quantity of iso-amyl alcohol (**Journal officiel N°67 du 12/11/2014**).

Weigh 3g of cheese, cross it in the butyrometer, Place the butyrometer in the water bath for 5min at $(65 \pm 2^\circ\text{C})$, Remove the butyrometer rom the water and shake for 10s Repeat the last two operation until the proteins are dissolved, Remove the butyrometer from the water bath and add 1ml of iso-amyl alcohol shake for at least 3s, Add the sulfuric acide Shake the butyromètre for 10s, as a soon the fat has risen in the butyrometre chamber, turn it over again so that the acid flows out of the stem, Place the butyromètre in the water bath for 5min.

Remove the butyromètre from the water bath, centrifuge at a relative centrifugal acceleration of (350 ± 50) g for 10min, Place the butyromètre in the water bath for 5min Remove the butyromètre from the water bath .The reading should be made to the nearest half step (25%).

Expression of the results as follows: **B-A**

A: this is the reading taken at the lower end of the fat column.

B: this is the reading taken at the upper end of the fat column.

6) Determination of the dry extract

Drying of a weighed test sample and mixing with sand by heating in an oven set at 102°C.

Weigh the dried test sample to determine the mass loss (**Journal officiel N°25 du 04/05/2014**).

Place 3g of the cheese on a surface of the capsule, Heat the capsule with its lid placed next to it, in the oven set at 102°C, and then dry the contents for 3hours. Mix the test sample and sand thoroughly and distribute the mixture evenly. Put the lid back on the capsule and let it cool down in the desiccator to room temperature .Reheat the capsule with its lid for 1hour, put the lid back on the capsule and let it cool down to room temperature in the desiccators, Weigh the capsule with its lid to the nearest 1mg and record the mass with 4 decimal places.

Repeat the last procedure until you observe between two successive weightings a decrease in mass less than or equal to 2 mg or an increase in mass, Record the minimum mass of the capsule.

Expression of the results as follows:

$$Wt = \frac{(m_2 - m_0) - (m_3 - m_4)}{m_1 - m_0} \times 100\%$$

- m1: the mass in g of the prepared capsule.
- m2: the mass in g of the test sample and the capsule before drying.
- m3: the mass in g of the capsule used for the blank test.
- m4: the mass in g of the capsule prepare.

7) Determination of crude protein

According to (Lecoq et al., 2021) in order to determine the amount of protein contained in the cheese samples we proceeded to the determination of total nitrogen by the Kjeldahl method. This method is based on the transformation of organic nitrogen into ammonium sulfate under the action of sulfuric acid in the presence of a catalyst. The latter is carried out in three phases : digestion (mineralization), distillation and titration (Iso 8968-1 :2001) .

➤ Mineralization

In a Kjeldahl flask, 0.5 g of a ground cheese sample, 2 g of a catalyst (selenium, potassium sulfate and copper sulfate) and 20 ml of concentrated H₂SO₄ (97%).concentrated (97%). This mixture has a black color.

Then the heat the matra until the black color turns into a clear color, at which point the organic nitrogen is clear color, at which point the organic nitrogen is transformed into mineral nitrogen. After cooling, transfer the mineralized sample to a vial and adjust the volume to 100 mL with distilled water.

➤ Distillation

This is carried out in a Buchi B-324 distillation unit. Introduce in a flask, 20mL of the contents of the flask, 50 mL of distilled water and 50 mL of the soda (40%). In parallel, add 20 mL of boric acid (H₃BO₃) (4%) with a few drops of color indicators (methylene red and methylene blue). The distillation is stopped after 4 minutes from the beginning of boiling.

➤ Titration

As boric acid was used as recovery solution, the excess of borate anions is then titrated with sulfuric acid (0.02N) until the color change from green to pink-violet. The total nitrogen is calculated according to the following formula:

$$N \% = ((V1-V0) \times 0.28) / P_{essai} \times 100$$

N%: Percentage of nitrogen.

P%: Percentage of protein.

V1: Volume of the concentrated sulfuric acid (mL).

V0: Volume of concentrated sulfuric acid used for the control (mL).

P test: The mass of the test sample (g).

The total nitrogen content is converted to crude protein content according to the following formula:

$$\text{Crude protein content (\%)} = \text{N total (\%)} \times 6,25$$

Where 6.25 is a conversion factor based on the average protein nitrogen content.

8) Determination of salt

The Mohr's method is used for the determination of salt, for that the chlorides are determined in neutral medium by titrated solution of silver nitrate in the presence of potassium chromate. The end of the reaction is indicated by the appearance of the characteristic red color of silver chromate (**Journal officiel N°17 du 18/03/2018**).

Weigh 2g of cheese ,Add 25ml of distilled water and shake to dissolve the cheese Add 2ml K^+ 15% ferrocyanid and 2 ml zin acetate 30% (Deification) and let stand for 15min . Pouring 25ml of the filtered solution into an Erlenmeyer flask and make up to 100 ml with ditilled water .Add 1ml of 5% K^+ chromate, Titrate with 0.1N silver nitrate $AgNO_3$.

Expression of the results is as follows:

$$\text{NaCl \%} = \text{cb} * \text{N} * \text{f} * (100 / \text{PE}) * \text{invdill} * (\text{Meq NaCl}/1000)$$

Cb: burette drop

N: normality

F: correction factor.

Pe: test sample.

Invdill: inverse dilution.

MeqNaCl: equivalent mass NaCl (58.5g/mol).

I.2.6. Microbiological analysis of cheese

The microbiological analyses were carried out according to the protocols of the executive decree of 02 July 2017 of the official journal of the Algerian Republic. This last aims on the one hand to preserve the organoleptic and sensory characteristics of the product, therefore to extend its shelf life and to prevent food poisoning due to the presence of pathogenic microorganisms before transmission to the consumer (Giraud et al., 2012).

1) Preparation of the stock solution and decimal dilutions

Under aseptic conditions, weigh 10 g of test sample of cheese made directly into the container. Add 90 ml of Tryptone salt-water diluent at pH (7.5 ± 0.2). Blend until the product is well homogenized (1 minute to 3 minutes). A series of decimal dilutions is performed by taking 1 ml of the mother solution in 9 ml of physiological water which constitutes the dilution (10^{-1}), after homogenization of the latter, the same operation is repeated for the successive dilutions in order to prepare the appropriate number of decimal dilutions for the counting of each flora (JORA N°38, 2014).

2) Identification of Escherichia coli

The culture medium used is agar a la bile, red neutral, violet crystal and lactose (VRBL), which inhibits the growth of gram-positive bacteria, as well as gram-negative bacteria. The petri dishes are seeded with sterile pipettes. With 1 ml of dilution 10^{-1} , the second box of dilution 10^{-2} . Poured 15 ml on the petri boxes of the VRBL agar. Carefully mix the inoculums in the culture medium and let the mixture stand solidify by placing the petri dishes on a cold, horizontal surface. After solidification of the mixture, add a layer of about 5 ml of the VRBL agar also mix carefully as described at the beginning to prevent spreading of colonies. Reading is done after 24-48 hours incubation at 44°C by counts of purple red colonies with a diameter greater than or equal to 0,5 mm. (JORA N°75, 2017).

3) Identification of coagulase positive staphylococci

Surface inoculate 1 ml of the stock solution on Baird Parker agar poured onto two petri dishes. Under the same conditions, inoculate decimal dilutions obtained from the test sample or the stock suspension, at the rate of two dishes per dilution. Incubate dishes at 35°C or 37°C for 18 h to 24 h and, if necessary, an additional 24 h from the number of characteristic colonies per Petri dish, calculate the number of coagulase positive staphylococci per mL or per gram of test sample (JORA N°68, 2014).

4) Identification of Salmonella

- **Pre-enrichment**

25g of each fresh cheese sample is put in 225 ml of TSE. After 30 min 2.25 ml of an aqueous solution of bright green is aseptically introduced into each sample. Mix thoroughly. Incubate in the oven at 37° C for 20 ± 2 hours.

- **Enrichment**

Add 10 ml of the pre-enriched mixture to 100 ml of selenite-cystine broth and incubate at 37°C ± 1 for 48 hours.

- **Isolation**

After incubation, perform stratum isolations on the surface of two solid selective media preferably cast in Petri dishes. Use Hektoen agar then return the plates to the oven at 37° C for 18 to 20 hours if the development is insufficient to continue the incubation.





Salmonella are presented as blue-green colonies with a black center on Hektoen agar (NA 2688).

5) Identification of *Listiria monocytogenes*

The detection of *Listeria monocytogenes* requires a pre-enrichment of 25g of food in 225ml of Fraser selective pre-enrichment broth, homogenized with a Stomacher type homogenizer. After 4 hours, half a Fraser supplement is added. Once incubated for 24 hours at 37°C. 0,1ml of the pre-enrichment medium is inoculated into test tubes containing 10 ml of Fraser broth. After incubation at 37°C for 48h, the broths with a positive reaction take on a dark coloration which can be black or dark brown while those with a negative reaction retain the straw coloration of the freshly prepared broth. A 0,1ml volume of the enrichment broth was placed on selective agar (PALCAM or OXFORD) and incubated at 37°C and observed for 48 hours. Suspect colonies were purified on tryptone soy agar with yeast extract (TSA-YE) and incubated for 24 hours at 37°C (ISO 11290-1).

The following table summarized all the microbiological analyses performed:

Table III: Microbiologic criteria and procedure of the germ search according to the standard:

| Searched germs | Levy | Culture medium | Seeding | Incubation | Description | | Standard |
|-------------------------------|---|----------------|----------------|--------------|--|-------------------------------------|--|
| Escherichia coli | 1ml of the stock solution 1ml of the 1/10 dilution | VRBL | On the surface | 44 °C/ 24h |  | pink to purple colonies with halo | JORA N°75, 2017 |
| Staphylococcus Aureus | 1ml of the stock solution | Baird-Parker | On the surface | 37°C/48h |  | Black Colony with halo | JORA N°65 art 2019/ art, 2014 |
| Listeria monocytogenes | 25g of the stock solution | Oxford | En masse | 37°C/24h |  | Black Colony with halo | ISO 11290-1 |
| Salmonella | 25g of the stock solution | Hecktoen | On the surface | 37°C/24h-48h |  | Green blue colony with black centre | NA 2688 JORA N°44, 2017 |

1.2.7. sensory evaluation of cheese

The sensory evaluation was carried out at the level of the sensory analysis laboratory of the University of Bejaia. Two types of analysis were carried out:

- A sensory analysis for which we called up on the expert panel that we trained during this study.

- A hedonic analysis that we used a consumer panel of 120 naive individuals

Five samples of cheeses (Enriched and no enriched cheeses) were presented for each taster with a questionnaire to be completed see (appendix).

The various samples presented are as follows:

- **Cheese A:** not enriched
- **Cheese B:** enriched in *Nigella sativa* L. oil;
- **Cheese C:** enriched with *Nigella sativa* L. seeds powder
- **Cheese D:** enriched with *Nigella sativa* L. seeds;

- **Cheese E:** enriched with *Nigella Damascena* L. seeds.

The data collected from the questionnaires distributed to the judges were processed using the software XL STAT version 2014, which is a complete tool for data analysis and statistics, involved in marketing studies and consumer behavior analysis. This software uses Microsoft Excel as an interface for data recovery and display of results. However, all mathematical calculations are performed outside of Excel. Access to the various modules is possible through menus and toolbars (**Addinsoft, 2013**).

The main features of this software used to interpret the results of the sensory evaluation performed are: Product characterization, after completing a Principal component Analysis (PCA), Agglomerative Hierarchical Clustering (AHC) and Preference MAPPING (PREFMAP).

The work carried out in the sensory analysis laboratory was aimed at developing and validating a panel of experts for sensory analysis. The setting up of this panel involved several steps:

- Recruitment and selection: according to the distributed questionnaire, out of the 50 people recruited, 27 were eliminated for various reasons such as: health problems, lack of availability, lack of motivation...
- In-depth selection: the SPENCER method adopted includes a total of 3 selection tests, after which a panel of 11 judges was formed.
- Training: The panel of 11 judges was selected to participate in a training session, analyzing a food, in our case they analyzed 5 samples of fresh cheese. The results obtained are that the judges preferred the cheeses seasoned with *Nigella damascena* L. seeds (67%) and *Nigella sativa* L. oil, followed by the cheese seasoned with *Nigella sativa* powder. These judges will be called to a meeting where the panel we have formed will join the old panel. In order to explain their roles and discuss all the difficulties and the mistakes made during the training sessions and during the analysis of the food products. This panel will also be called upon to continue regular training sessions in order to retain their sensory skills and complete their training.

During this study, we also learned the process of making fresh cheese prepared with 100% cow's milk. Five types of fresh cheese have been prepared (enriched and no enriched). Some photochemical analyses and a study of antioxidant activity were carried out for the seeds of *Nigella sativa* and *Nigella damascena* used for enrichment of cheese. The results obtained confirm that these seeds are quite rich in phenolic compounds and have a significant antioxidant activity, which can enrich the prepared cheeses. As well as to the improvement of organoleptic characteristics essentially the aroma.

The results of the physico-chemical analyses of fresh cheese show that it is a good source of total dry extract (40.7%) and fat (20.5%), quite rich in protein (13.2%), but not rich in salt (0.68%) and carbohydrates (0.1%).

The results of the microbiological analyses show that this cheese has a good microbiological quality, which makes them suitable for consumption.

The results of the sensory evaluation, show that cheeses seasoned with seeds of *Nigella damascene* and *Nigella sativa* oil are more appreciated (67%), by their aroma and tartinability. Followed the cheese seasoned with *Nigella sativa* powder (56%). In the end, the least preferred (33%) cheeses are not enriched cheese (A) and seasoned with *Nigella sativa* seeds (D).

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Annex I: Recruitment questionnaire an expert panel of generalists

Recruitment questionnaire

“Setting up an expert panel of generalists”

Surname and given name: **Telephone**

Age: **Function:** **Address (Region):**

Email:

1. Have you ever heard of sensory analysis? Yes No

2. Can you give us some parameters studied in sensory analysis?

.....

3. Have you ever heard of a sensory assessment group? Yes No

4. Have you ever participated in a sensory assessment group? Yes No

5. Availability:

a. Are there days you were available regularly? Yes No

6. What weekdays may be available?

7. Health: Take – drugs that affect your senses, especially your

Taste and your workshop? Yes No

8. Do you smoke? Yes No

9. Do you have?

Oral Conditions Food Allergies Hypertension Diabetes

10. Eating Habits:

a. What is (are) the food you like least?

b. What is (are) your favourite food(s)?

c. What food can't you eat?

d. What food do you not like?

For more information: labo.analysesensorielle@yahoo.fr

Thank you for your participation

Annex II: Dilution concentration of each flavour (arithmetic series)

| Dilution | Concentration (g/L) |
|-------------------------------|--------------------------------------|
| 1st Concentration | 173ml of the prepared stock solution |
| 2 nd Concentration | 150ml of the prepared stock solution |
| 3rd Concentration | 125ml of the prepared stock solution |
| 4th Concentration | 100ml of the prepared stock solution |
| 5th Concentration | 75ml of the prepared stock solution |
| 6th Concentration | 50ml of the prepared stock solution |

Table I: Dilution concentration of each flavour (arithmetic series)**Annex III:** List of Substances for the Odour Identification Test

| Pure body | Essential oils | Aromas |
|-----------------|----------------|----------------|
| 1- Phenol | 6- Cinnamon | 14- strawberry |
| 2- Ammonia | 7- Lemon | 15- vanilla |
| 3- Benzaldehyde | 8- Eucalyptus | 16- cherry |
| 4- Butyric acid | 9- Rosemary | 17- Banana |
| 5- Acetic acid | 10- Lentistics | 18- caramel |
| | 11- Clove | 19- honey |
| | 12- Thyme | 20- pistachio |
| | 13- Lavender | |

Table II: List of Substances for the Odour Identification Test

Annex IV: Hedonic analysis questionnaires for fresh cheese (consumer panel)**Hedonic analysis questionnaires for fresh cheese (consumer panel)**

Gender: M or F **Age:** **Position No:** **Date:**

5 coded fresh cheese samples are presented to you, you are asked to evaluate the different organoleptic characteristics by assigning a score between 1 and 5 according to the scale presented.

NB: Please rinse the mouth after each tasting of a sample.

Do you like the color?

1-Not appreciated

2-Little appreciated

3-Moderately appreciated

4-Well appreciated

5-Very appreciated

| Sample A | Sample B | Sample C | Sample D | Sample E |
|-------------|-------------|-------------|-------------|-------------|
| | | | | |

Do you like the smell?

1-Not appreciated

2-Little appreciated

3-Moderately appreciated

4-Well appreciated

5-Very appreciated

| Sample A | Sample B | Sample C | Sample D | Sample E |
|-------------|-------------|-------------|-------------|-------------|
| | | | | |

Do you enjoy the taste?

1-Not appreciated

2-Little appreciated

3-Moderately appreciated

4-Well-liked

5-Very appreciated

| Sample A | Sample B | Sample C | Sample D | Sample E |
|-------------|-------------|-------------|-------------|-------------|
| | | | | |

Overall preference

Assign a score of 1 to 9 to each sample based on your preference, with 1 being the least preferred sample and 9 being the most preferred sample. As shown in the scale below:

1. Extremely unpleasant

2. Very unpleasant

3. Unpleasant

4. Somewhat unpleasant

5. Neither pleasant nor unpleasant

6. Quite nice

7. Enjoyable

8. Very nice

9. Extremely enjoyable

| Sample A | Sample B | Sample C | Sample D | Sample E |
|---------------------|---------------------|---------------------|---------------------|---------------------|
| | | | | |

Thank you for your contribution

Annex V: Fresh cheese sensory analysis questionnaires (expert panel)**Fresh cheese sensory analysis questionnaires (expert panel)****Last Name First Name:****Gender: M or F Age: Position No: Date:**

Five samples of coded fresh cheese are presented to you. You are asked to evaluate the different organoleptic characteristics by assigning a score between 1 and 5 according to the scale presented.

NB: Please rinse the mouth after each tasting of a sample and do not swallow to avoid the phenomenon of satiety, except for the question of preference.

1. Color:

1. White
2. Beige
3. Dark Beige
4. Black
5. Dark Black

| Sample A | Sample B | Sample C | Sample D | Sample E |
|-------------|-------------|-------------|-------------|-------------|
| | | | | |

2. Smell:

1. Absent
2. Low
3. Average
4. Strong
5. Very strong

| Sample A | Sample B | Sample C | Sample D | Sample E |
|-------------|-------------|-------------|-------------|-------------|
| | | | | |

3. The aroma:

1. Absent
2. Low
3. Medium
4. Strong
5. Very strong

| Sample A | Sample B | Sample C | Sample D | Sample E |
|-------------|-------------|-------------|-------------|-------------|
| | | | | |

4. Salty Tast

1. Absent

2. Low

3. Medium

4. Strong

5. Very strong

| Sample A | Sample B | Sample C | Sample D | Sample E |
|-------------|-------------|-------------|-------------|-------------|
| | | | | |

5. Acid taste

1. Absent

2. Low

3. Medium

4. Strong

5. Very strong

| Sample A | Sample B | Sample C | Sample D | Sample E |
|-------------|-------------|-------------|-------------|-------------|
| | | | | |

6. Back taste

1. Absent

2. Low

3. Medium

4. Strong

5. Very strong

| Sample A | Sample B | Sample C | Sample D | Sample E |
|-------------|-------------|-------------|-------------|-------------|
| | | | | |

7. Texture1. Very weakly
grainy

2. Weakly grainy

3. Medium
(neither granular
smooth)

4. Smooth

5. Very smooth

| Sample A | Sample B | Sample C | Sample D | Sample E |
|-------------|-------------|-------------|-------------|-------------|
| | | | | |

8. Consistency

1. Very Mole

2. Mole

3. Average

4. Firm

5. Very firm

| Sample A | Sample B | Sample C | Sample D | Sample E |
|-------------|-------------|-------------|-------------|-------------|
| | | | | |

9. Tartinability

1. Very difficult

2. Difficult

3. Average

4. Easy

5. Very easy

| Sample A | Sample B | Sample C | Sample D | Sample E |
|-------------|-------------|-------------|-------------|-------------|
| | | | | |

10. Preferences**Do you like the color?**

1-Not appreciated

2-Little
appreciated3-Moderately
appreciated

4-Well appreciated

5-Very appreciated

| Sample A | Sample B | Sample C | Sample D | Sample E |
|-------------|-------------|-------------|-------------|-------------|
| | | | | |

Do you like the smell?

1-Not appreciated

2-Little
appreciated3-Moderately
appreciated

4-Well appreciated

5-Very appreciated

| Sample A | Sample B | Sample C | Sample D | Sample E |
|-------------|-------------|-------------|-------------|-------------|
| | | | | |

Do you enjoy the taste?

1-Not appreciated

2-Little appreciated

3-Moderately appreciated

4-Well-liked

5-Very appreciated

| Sample A | Sample B | Sample C | Sample D | Sample E |
|----------|----------|----------|----------|----------|
| | | | | |

Overall preference

Assign a score of 1 to 9 to each sample based on your preference, with 1 being the least preferred sample and 9 being the most preferred sample. As shown in the scale below:

1. Extremely unpleasant

2. Very unpleasant

3. Unpleasant

4. Somewhat unpleasant

5. Neither pleasant nor unpleasant

6. Quite nice

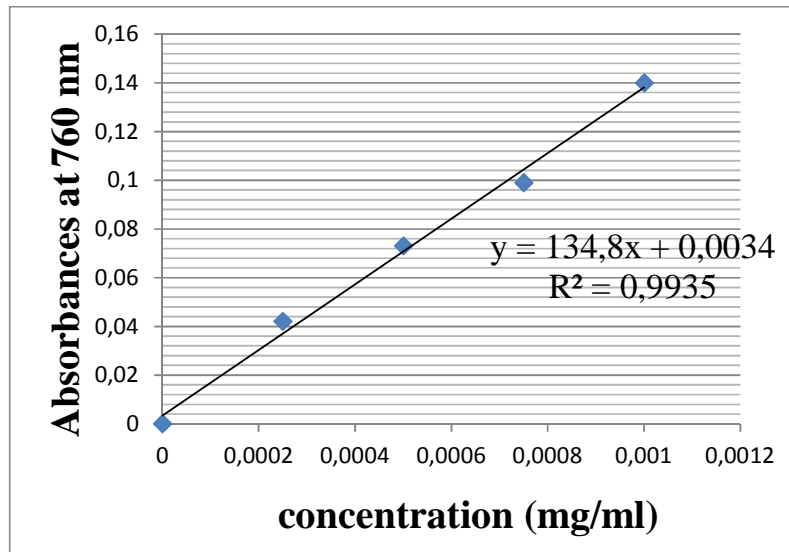
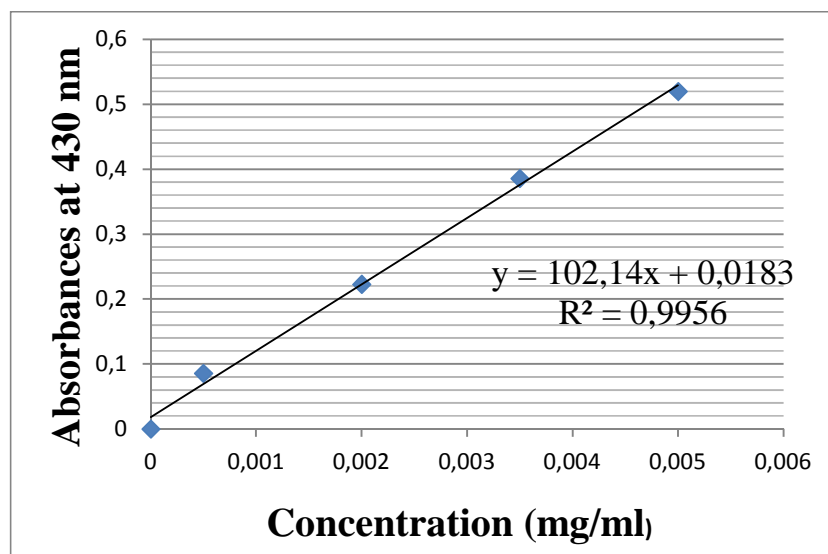
7. Enjoyable

8. Very nice

9. Extremely enjoyable

| Sample A | Sample B | Sample C | Sample D | Sample E |
|----------|----------|----------|----------|----------|
| | | | | |

Thank you for your contribution

Annex VI: Phenolic Compound Deferent Assay Calibration Curves**Figure 1:** Calibration curve for the determination of total polyphenols.**Figure 2:** flavonoid assay calibration curve

Annex VII: the results of the sensory evaluation Product characterization, after completing a Principal component Analysis (PCA).

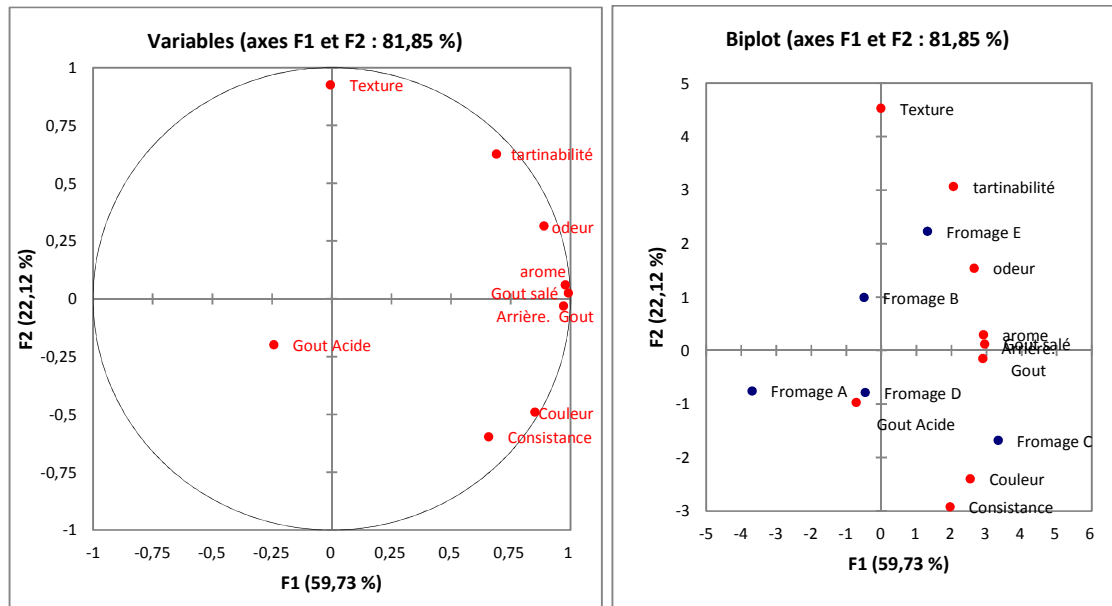


Figure 3: Correlation between variables and factors and coordinates of observations (PCA).

Annex VIII: the results of the sensory evaluation Agglomerative Hierarchical Clustering (AHC).

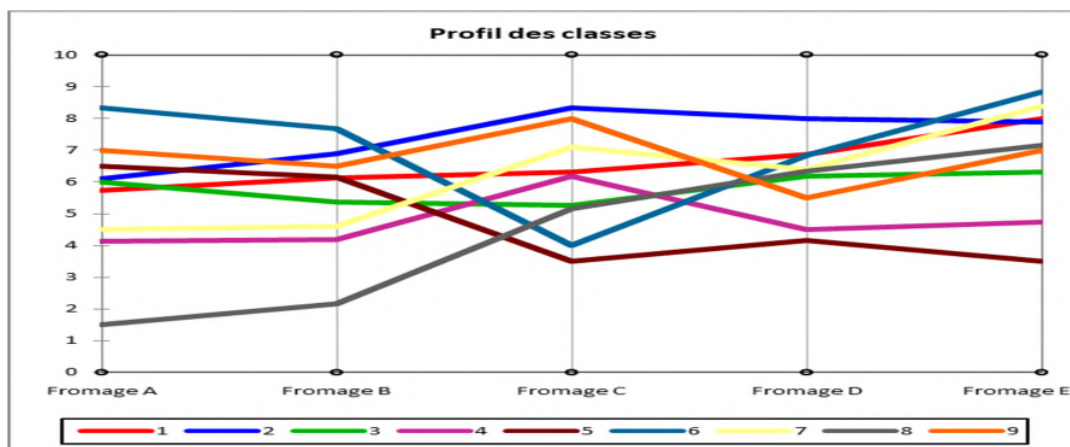


Figure 4: Profile of created classes.

Abstract

This study was conducted with the purpose of forming an expert panel of generalists for sensory evaluation, at the laboratory of sensory analysis of the University of Bejaia.

The method of Spencer was followed for the selection and training of judges. At the end of the selection steps, 11 judges were trained in the sensory analysis of foods by performing the sensory evaluation of 5 samples of fresh cheese that we have developed during this work.

The results obtained showed that the cheeses formed have good physicochemical, microbiological and sensory qualities. The cheeses most appreciated by the judges are those enriched.

Keywords: expert panel, sensory analysis, Spencer method, fresh cheese.

Résumé

Cette étude a été effectuée dans le but de former un panel expert de généralistes pour l'évaluation sensorielle, au niveau du laboratoire d'analyse sensorielle de l'université de Bejaia.

La méthode de Spencer a été suivie pour la sélection et l'entraînement des juges. A la fin de la phase de sélection, 11 juges ont été formés, ils étaient entraînés à l'analyse sensorielle des aliments en effectuant l'évaluation sensorielle de 5 échantillons de fromage frais que nous avons élaborés au cours de ce présent travail.

Les résultats obtenus ont montré que les fromages formés sont de bonnes qualités physico-chimiques, microbiologiques et sensorielles. Les fromages les plus appréciés par les juges sont ceux enrichis.

Mots clés : panel d'experts, analyse sensorielle, méthode Spencer, fromage frais.