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Mémoire de Fin de Cycle
En vue de l'obtention du diplôme

MASTER

Thème

Valorisation d'un sous-produit par l'extraction des composés bioactifs

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To all, thank you very much.

DEDICATION

Alhamdulillah and thanks for the beginning and the end

(وآخر دعواهم أن الحمد لله رب العالمين)

The journey was neither short nor the road fraught with facilities, but I did it, so praise be to Allah, who reached the end thanks to His grace.

I dedicate this success to my ambitious self-first who started with ambition and ended with success.

to my paradise, my sunshine to the source of my joy and happiness ,my moon and the thread of hope that lights my way, my half mom.

to the one who made me a woman, my source of life, love and affection, to my support who was always by my side to support and encourage me to my first love my father.

to my brother abdelmadjid for the love he serves me.

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ABSTRACT

This work proposes the optimization of the ultrasound assisted extraction (UAE) for total phenolic compounds (TPC) from olive pomace. The optimized extract was obtained using ethanol 52% (v/v) for 55 min at 60°C, and it was found to be rich in total phenolics (1106 ± 56.67 mg GAE/100g DW) and exerted a good antioxidant effect with IC50 of 103.5 ± 6.68 µg/mL, good anti-diabetic activity with IC50=20.44 ± 4.24 µg/mL, and good anti-inflammatory activity with IC50 of 823.36 ± 11.30 µg/mL. The experimental values are close to the predicted values, confirming the validity of the mathematical model obtained. Olive pomace was used for the fresh cheese formulation, and this supplementation did not impact its physicochemical properties however, the formulated fresh cheese presented a good acceptability, even better than the control.

Keywords: Olive pomace; Fresh Cheese; Response surface methodology; Antioxidant activity; Anti-diabetic activity; anti-inflammatory activity.

RÉSUMÉ

Ce travail propose l'optimisation de l'extraction assistée par ultrasons (UAE) pour les composés phénoliques totaux (TPC) du grignon d'olive. L'extrait optimisé a été obtenu en utilisant de l'éthanol 52% (v/v) pendant 55 min à 60°C, et il s'est avéré riche en composés phénoliques totaux (1106 ± 56,67 mg GAE/100g DW) et a exercé un bon effet antioxydant avec IC50 de 103,5 ± 6,68 µg/mL, une bonne activité antidiabétique avec IC50=20,44 ± 4,24 µg/mL, et une bonne activité anti-inflammatoire avec IC50 de 823,36 ± 11,30 µg/mL. Les valeurs expérimentales sont proches des valeurs prédites, ce qui confirme la validité du modèle mathématique obtenu. Le grignon d'olive a été utilisé pour la formulation du fromage frais, et cette supplémentation n'a pas eu d'impact sur ses propriétés physicochimiques ; cependant, le fromage frais formulé a présenté une bonne acceptabilité, encore meilleure que le contrôle.

Mots-clés : Grignons d'olive ; fromage frais ; méthodologie de la surface de réponse ; activité antioxydante ; activité antidiabétique ; activité anti-inflammatoire.

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List of abbreviations:

OP: olive pomace.

OO: olive oil.

TPC: total phenolic compound**.**

PC: phenolic compounds

EAG: equivalent acid galic

DP: dry powder

INTRODUCTION

Introduction:

Losses in the form of trash and byproducts have increased along with the growth of the fruit and vegetable processing industries. Although these processing leftovers are not commonly consumed, they contain valuable bioactive substances that are used in the creation of enhanced or functional foods, including phytochemicals and secondary metabolites **(Kumar K. S., 2021).**

Olives (Olea europaea L.) are widely cultivated in the Mediterranean region and are an important source of olive oil, widely appreciated for its nutritional qualities and health benefits. Besides oil, olive production produces a large number of by-products such as leaves, stems and pits, which are often considered waste. However, these by-products contain a large number of bioactive compounds, including phenolic compounds, which are attracting increasing attention due to their antioxidant, anti-inflammatory, anti-diabetic and anti-cancer properties **(Bouaziz, 2006).**

Phenolic compounds are a class of compounds found in olives and their byproducts that have many health-promoting properties. These compounds include hydroxytyrosol, caffeic acid, rosmarinic acid, and other flavonoids, which have been shown to have protective effects against cardiovascular disease, cancer, premature aging and more **(Omar, 2010).**

Ultrasonic extraction has become an increasingly popular technology for the recovery of phenolic compounds from olive by-products due to its high efficiency and environmental friendliness. This method uses ultrasound to cause cell walls to rupture, promoting the release of bioactive compounds into the extraction solvent. In addition, the application of ultrasound makes it possible to considerably reduce extraction time compared to traditional methods while maintaining the quality of the extract obtained **(Cvetanović, 2022).** this initiative aims to develop food items based on natural and organic resources and to provide value to the agri-food business.

The aim of this study is to obtain the optimum conditions for the extraction of phenolic compounds in the first place, and to valorize industrial olive waste in the second place by incorporating it into a fresh cheese, In order to answer a crucial question: Can industrial olive waste be used in food products?

BIBLIOGRAPHIC SYNTHESIS

Bibliographic synthesis

1 Generalites

1.1 Olive tree:

The olive tree has a history dating back to ancient times, when it was revered for its economic importance, nutritional benefits and religious symbolism. It is thought that the olive tree was cultivated as early as the 6th millennium BC, according to archaeological evidence; in Anatolia, which is present-day Turkey, its use gradually spread throughout the Mediterranean basin **(Besnard, 2013).**

The plant, a kind of small evergreen tree in the Oleaceae family, is primarily found in the Mediterranean region, although it can also be found in North and South America, Australia, and New Zealand. It has sturdy branches and grayish bark. The olive, a smooth, tiny, green or purple drupe, is its fruit and is used to make olive oil **(Simone Filardo, 2024).**

1.2 Olives:

1.2.1 Definition:

Olives are the fruit of the olive tree. Botanically speaking, they are smooth-skinned drupes with fatty flesh and a stone. Their oval shape is unmistakable and their color can vary from light green to deep black, depending on the stage of ripeness **(zubiria, 2020).**

1.2.2 Structure:

The fruit of the olive tree, the olive, is a fleshy, more or less oval drupe with a smooth skin. It is made up of three parts from the outside to the inside: The epicarp, the mesocarp and the endocarp (figure1) **(Fedeli, 1997).**

- **Epicarp:**

Made up of the epidermis and the cuticle, represents 1 to 3% of the weight of the fruit. It consists mainly of fatty acids, accompanied by alcohols and their esters, aromatic compounds and chlorophylls **(Cortesi N., 2000)**. Its color varies from green at the beginning of ripening, through green to yellowish, violet-pink, purple and black at full ripeness. These color variations are linked to the pigment composition of the fruit **(Bianchi G, 2003).**

- **Mesocarp:**

Also known as the flesh, represents 70 to 80% of the weight of the fruit. It contains an aqueous solution in an essentially protein matrix, the solutes of which are mainly sugars, accompanied by a number of organic acids, simple and complex phenols, either free or bound to the sugars, and liposoluble aroma compounds. The mesocarp contains most of the oil (96 to 98%), which is found in free form in the vacuoles and in bound form in the cytoplasm **(Cortesi N., 2000).**

- **Endocarp:**

Very characteristic of the variety, the endocarp (pit) represents 18 to 22% of the weight of the fruit. It is made up of two subsystems: the first is the outermost part of the seed and the second is the protein matrix containing the lipid and hydrophilic components **(Bianchi G, 2003).**

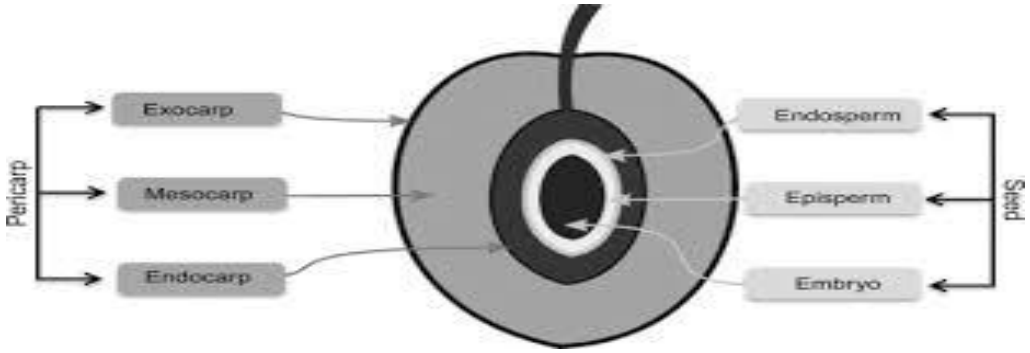


Figure 1: olive fruit structure **(Bianchi G, 2003).**

1.2.3 Chemical coposition:

The chemical composition of olives (Tables I) can vary depending on various factors such as the variety of olive tree, the stage of ripeness of the fruit and growing conditions. However, here are the main chemical components of olives:

Table I: Chemical composition of olive fruit **(COI, 1997).**

Components	Content
Water and liquid	65-72% of the weight of the fresh fruit
Lipids	17-30% of the weight of the fresh fruit
Protein	1.5% of the weight of the pulp
Sugar	12% of pulp weight
Organic acids	0.10-0.20% of pulp weight

Vitamins	Carotene: 0.15-0.23mg per 100g of pulp. Vitamin C: 12.9-19.1mg per 100g of pulp. Thiamine: 0.54-11.0mg per 100g of pulp. Vitamin E:238.1-352mg per 100g of pulp
Pigments	Caro Chlorophyll a and b Carotenoids Anthocyanin
Inorganic substances	potassium -chlorine Calcium -phosphorus Magnesium
Polyphenols and tannins	0.98% of fresh fruit weight

1.2.4 Nutritional value:

The very bitter taste of olives is due to oleuropein, which makes it impossible to eat fresh olives. Olives have to be processed to make them palatable and to remove the bitterness by converting them into olive oil, green or black table olives and other by-products. Olives are an important food because of their nutritional value; in addition to their beneficial fatty acids, particularly monounsaturated fatty acids, olives also contain minor components such as phenolic compounds and tocopherol **(Nacera, 2020).**

1.3 Olive pomace:

1.3.1 Definition:

Olive pomace (OP) is made up of crushed olive stone, olive husk and pulp, and water with a moisture percentage of around 60%. It is the primary byproduct of the manufacturing of olive oil, accounting for 35–40% of the processed olive's weight. Because of their high phytotoxicity, these by-products are regarded as being the most detrimental to the ecosystem, along with olive mill wastewater (OMWW) (Figure 3). Furthermore, OP has a rich mineral composition, squalene, a high lipid component, particularly in oleic acid (75% lipid content), and significant levels of cellulose (30%) and pectic polysaccharides (39%). Antioxidants like tocopherols and other phenolic compounds are also present in OP **(Raquel Rodrigues, 2023).**

1.3.2 Olive pomace types:

- Raw marc**:** These are the solid residues from the first extraction of olive oil. These grounds contain a lot of oil **(Ines & Radia, 2023)**
- Refined fruit pomace: obtained from raw fruit pomace by solvent extraction of residual oil. Depleted pomace is characterized by a low fat and moisture content **(Ines & Radia, 2023).**
- Sifted pomace**:** obtained through a sifting process to separate the crushed nut fragments from the pulp. Sifted pomace is described as "fat" if it has not been subjected to solvent extraction of the oil, and as "skimmed" and "skimmed" if it has been processed to remove.

1.3.3 Phenolic compounds in olive pomace:

Olive pomace is considered a rich source of phenolic compounds with diverse biological activities. It is rich in phenolic compounds such as: nüzhenide, hydroxytyrosol, tyrosol, oleuropein, caffeic acid, benzoic acid and rutin. **(Youdas, 2022).**

1.4 Olive paste:

Olive paste is obtained from the liquid fraction of the olives and water, which may be added during the crushing process. During this process the quality and quantity of the olive paste depends on the olive oil extraction process. They are also influenced by the variety of olive, the ripeness of the fruit and the climatic conditions **(Lamia, 2022).**

2 Phenolic compounds:

2.1 Definition:

Phenolic compounds (PC) are olive oil (OO) and olive pomace's (OP) primary bioactive components. the chemicals in olive oil have a role in their antioxidant, antidiabetic, anticancer, hypolipidemic, neuroprotective, cardioprotective, and antibacterial characteristics. The most prominent PC found in olive fruit are the secoiridoid compounds, specifically oleeuropein (Ole) and its aglycone form, as well as the byproducts tyrosol (Tyr) and hydroxytyrosol (HT), which are produced during its hydrolysis. 30% of the PC in olive fruit is made up of Tyr and HT, which have bioactive characteristics comparable to those of the original substances **(Luana Schmidt a, 2023).**

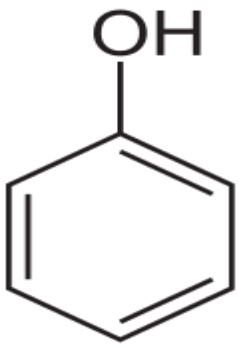


Figure 2: phenol structure **(Luana Schmidt a, 2023).**

2.2 The main phenolic compounds:

2.2.1 Secoiridoids:

The primary phenolic compounds found in raw olives consist of specific secoiridoids originating from oleosides, formed by the union of elenolic acid and a glucose residue. Repeatedly identified as the principal secoiridoids within the fruit are oleuropein, an ester of elenolic acid with 3, 4-dihydroxyphenethyl alcohol (hydroxytyrosol), demethyloleuropein, the acid form of oleuropein, and ligstroside, an ester of elenolic acid with 4-hydroxyphenethyl alcohol (tyrosol). Additionally, other derivatives of oleuropein are present in olive fruit, including oleuropein aglycon, hydroxytyrosilelenolate, enololeuropeindiale, as well as various glucosides such as hydroxytyrosol-1-O-β-glucoside, tyrosol-1-O-β-glucoside, hydroxytyrosol-3'-O-β-glucoside, hydroxytyrosol-4'-O-β-glucoside, and verbascoside, a caffeoylrhamnosyl glucoside of hydroxytyrosol **(Lounes, 2022).**

2.2.2 Phenolic acids:

Hydroxyderivatives of benzoic, cinnamic, phenylacetic, and phenylpropionic acids, including compounds like p-hydroxybenzoic, protocatechuic, vanillic, syringic, o- and p-coumaric, caffeic, chlorogenic, ferulic, sinapic, p-phenylacetic, 3,4-dihydroxyphenylacetic, homovanillic, and dihydrocaffeic acids, are found in varying levels within olives, contingent upon the specific variety of fruit **(D. Boskou, 2005).**

a.vanilic acid **b.galic acid**

Figure 3:vanilic acid and galic acid structure **(Luana Schmidt a, 2023).**

2.2.3 Flavonoids:

In olive fruit, you'll find flavonoids such as flavones, primarily luteolin, flavone and flavonol glucosides, predominantly rutin and luteolin 7-glucoside, and anthocyanins, mainly cyanidin 3-glycosides. The levels of luteolin 7-glucoside and rutin vary according to the ripeness of the fruit **(D. Boskou, 2005).**

a.Quercetin (flavonols). **b.Catechin (flavonols).**

Figure 4: flavonoids structure **(Luana Schmidt a, 2023).**

2.2.4 Phenolic alcohols:

In addition to secoiridoids, phenolic alcohols like tyrosol (p-HPEA) and hydroxytyrosol (3, 4-DHPEA), along with their derivatives, significantly contribute to the nutritional benefits of olive oil, a fact supported by an EFSA health claim. These compounds, simpler in structure compared to secoiridoids, are found in both olive fruits and oils **(Giulia vicario, 2023).**

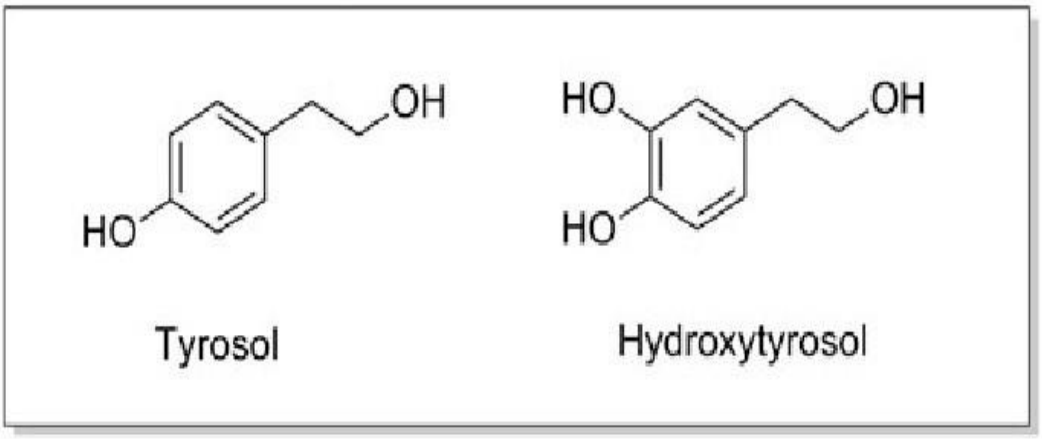


Figure 5: some phenolic alcohols structure **(Luana Schmidt a, 2023).**

2.2.5 Lignans:

Lignans are phenolic substances found in all kinds of plants, including olives and their by-products. Lignans include compounds such as pinoresinol and acetoxypinoresinol in olive pomace **(Bendini A., 2007)**.

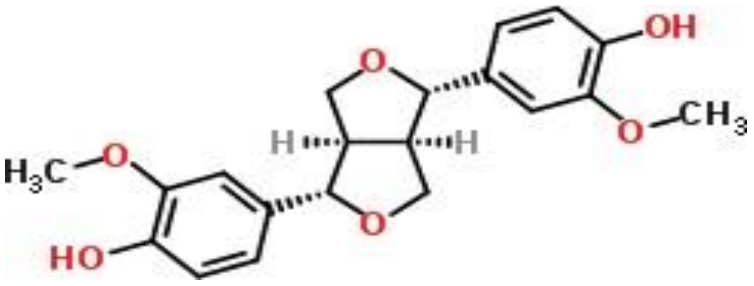


Figure 6: lignans structure **(Luana Schmidt a, 2023).**

2.3 Role of phenolic compounds:

The role of phenolic compounds in various aspects of plant life and human use of plants is now widely recognized:

*****in certain areas of plant physiology (lignification, growth regulation, molecular interactions with certain symbiotic or parasitic microorganisms, etc.).

*****in interactions between plants and their biological and physical environment (interactions with bacteria, fungi, insects, UV resistance), whether directly in nature or during the post-harvest preservation of certain plants.

*****in the quality criteria (color, astringency, bitterness, nutritional quality...) that guide human decisions regarding the consumption of plant organs (fruit, vegetables, tubers...) and the products derived from them through processing.

***** When certain characteristics of plants are modified during technological processing (such as the preparation of fruit juices, fermented drinks, etc.), where enzymatic browning that alters the quality of finished products frequently occurs.

*****to protect humans against certain diseases, thanks to their interaction with numerous enzymes and their antioxidant properties **(Jean Jacques Machiex, 2005)**

3 Ultrasound method:

Extraction is considered to be the first step in separating the desired bioactive compounds from the raw material, which can be processed by various methods and techniques. Extraction can be described as a mass transfer phenomenon in which soluble solids, contained in plant structures, migrate into the solvent until equilibrium is reached **(Hidayat, 2021).**

3.1 Ultrasound-assisted extraction:

Ultrasound is considered to be a versatile energy which is successfully used in a number of fields. Ultrasound is a form of energy or mechanical waves associated with sound at frequencies above those detected by the human ear (20 Hz to 20 kHz) **(Kumar, 2021).** Ultrasonic extraction is a technique used to extract natural compounds. The use of ultrasound enables extractions to be carried out in a short time (a few minutes) with a high degree of reproducibility **(Chemat, 2011)**. Ultrasound disrupts cell wall structures, inducing cell lysis and accelerating molecular diffusion across membranes, breaking down cell membranes **(Bourgou, 2016).**

Principle:

Ultrasound is an acoustic wave generated by a transducer through an inverse piezoelectric effect. They involve so-called cavitation phenomena, corresponding tothe formation of gas bubbles in a liquid, when the pressure at a given point in the liquid falls below its saturation vapor pressure **(Mason, 1988).**The cavitation bubbles thus formed are then subjected to the ultrasonic wave, which

causes them to oscillate, grow, resonate and implode. This phase generates, in highly localized areas, temperatures of around 5000 K and pressures of around 1000 atmospheres, which constitutes the hot-spot theory **(Suslick, 2001).**the propagation of these ultrasonic waves in a liquid medium is not continuous, and leads to a succession of compressions and depressions around a mean value. The organization of the reaction medium is disrupted by the wave, which can even cause the cohesion of the medium to break down. **(Suslick K. , 1989).**

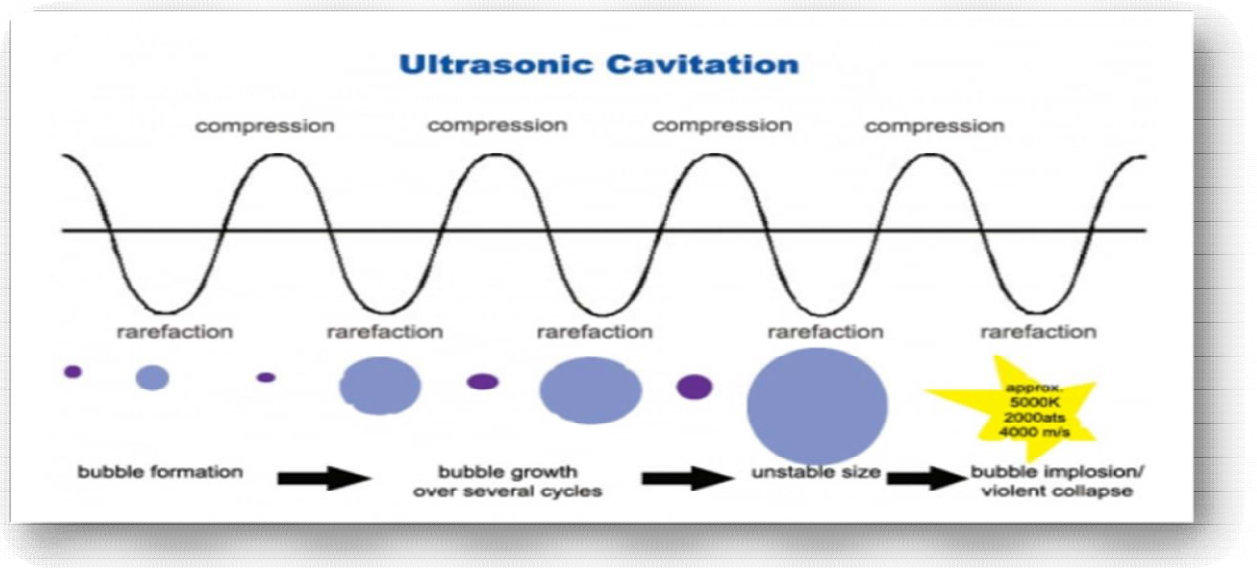


Figure 7: Acoustic wave propagation in a liquid medium **(Suslick K. 1989)**

3.2 Factors influencing ultrasound assisted extraction:

3.2.1 Choice of solvent:

The nature of the solvent is a very important parameter for ultrasonic cavitation, as its physical properties such as viscosity and surface tension, influence the efficiency of ultrasonic cavitation. In fact, low solvent viscosity and surface tension help to lower the cavitation threshold (Blake's threshold), since cohesive forces are lower **(Assami, 2014).**

3.2.2 Ultrasonic bath frequency:

The parameter of sonication frequency holds great significance. The frequency determines how big of a vacuum must be in order to cross the cavitation threshold. The duration of the

depression is shorter the higher the sonication frequency. Cavitation is more challenging at higher frequencies. The frequencies that are most frequently employed are 20–40 kHz.

3.2.3 Volume of solvent:

The amount of solvent required for a single sample is often between 10 and 30 ml. In some cases, solvent volume can be an important parameter for efficientfor efficient extractions. The volume of solvent must be sufficient to ensure that the entire sample is immersed, particularly in the case of a matrix that will swell during the extraction process **(Eskilsson, 2000)**.

3.2.4 Extraction temperature:

Several studies have shown that temperature has a significant effect on ultrasonic cavitation. An increase in medium temperature leads to a change in medium viscosity and a rise in saturated vapor pressure, which facilitates cavitation. This facilitates cavitation, but the bubbles implode less violently. This is why most sonochemical reactions are carried out at low temperatures **(Mason T. J., 1999).**

3.2.5 Extraction time:

Time is another important factor influencing the ultrasonic extraction process.

3.3 Ultrasound applications:

The use of ultrasound for the extraction of plant or food matrices is a new tool for increasing yields and/or accelerating extraction kinetics. These improvements can be attributed to enhanced diffusion of dissolved substances from inside the cell to the extraction medium. The first applications were linked to the determination of metals in foodstuffs. Today, applications cover the extraction of many other compounds, such as aromas, antioxidants, oils and colorants.

3.4 Advantages:

Ultrasound-assisted extraction has several advantages Including:

*High yields.

*Solutes diffuse more rapidly into the extraction medium, reducing extraction time

*Extraction temperature is lower, making it possible to extract substances that are thermo sensitive.

4 Cheese

4.1 Definition:

According to the French decree of December 30 1988, "the name 'cheese' is reserved for fermented product, ripened or not, obtained from the following materials: milk, partially or totally skimmed milk or skimmed milk, cream, fat, buttermilk, used alone or in a mixture coagulated in whole or in part before draining or after partial removal of the aqueous phase. Cheese is obtained by coagulation of milk using rennet or other coagulating agents".

4.2 Types of cheese:

There are several types of cheese:

4.2.1 Fresh cheeses:

These are drained cheeses obtained by centrifugation or filtration. They are essentially undergoing malolactic fermentation (but most often with a light rennet) and have not matured (no ripening). They have a high moisture content (70 to 75%); examples: petit swiss, demi-sel cheese, etc **(J.P, Microbiologie alimentaire, 2003).**

4.2.2 Soft cheeses:

These cheeses are obtained by the action of rennet, after ripening and malolactic fermentation. But the paste is neither cooked nor pressed. Draining is a slow process, involving simple cutting and if necessary beating. Their humidity is medium (50 to 55%). Their protection is improved by cooling. A distinction is made between:

- Soft "moussée" cheeses, generally with a moldy rind (Camembert, Brie, carré de l'Est)
- Soft, washed-rind cheeses (Munster, Livarot, Pontl'Evêque, etc.).
- Soft blue-veined cheeses (Roquefort and other "bleus", etc.). blue cheeses, etc.) **(J.P, Microbiologie alimentaire, 2003).**

4.2.3 Pressed cheeses:

These cheeses are obtained by the action of rennet. After ripening by malolactic fermentation, the curds are cut, drained, stirred and pressed. Their moisture content is medium (45 to 50% for uncooked cheeses) or low (35 to 40% for cooked or highly stirred). Their preservation is enhanced by cold. A distinction is made between:

- Firm uncooked cheeses (pressed and ground) (cantal, etc.);
- Uncooked pressed cheeses with washed rind (St Paulin, Reblochon, etc.);
- Uncooked pressed cheeses with a moldy rind (St Nectaire, Tomme de Savoie, etc.);
- Uncooked pressed cheeses with artificIal rind (Edam, etc.);
- Pressed-cooked cheeses with openings (Emmenthal, Comté, etc.);
- Pressed-cooked cheeses without opening (Beaufort, etc.);
- Very hard pressed cheeses (Cheddar, etc.) **(J.P, Microbiologie alimentaire, 2003)**

5 Fresh cheese:

5.1 Definition:

For consumers, the term fresh cheese vokes the notion of an unripened product with a relatively short shelf life, stored at low temperature**.**

Fresh cheeses result from the slow coagulation of milk by acidification, with or without the combined action of a small quantity of rennet. Fresh cheeses vary widely according to the degree of coagulum draining and the fat content of the milk used.

5.2 Characteristics of fresh cheese:

Fresh cheeses are easily recognized by their whiteness, their generally shiny appearance and their lack of rind. In addition to their common characteristics, there is considerable diversity, particularly in terms of texture **(Harbutt., 2010)**

5.3 Nutritional value:

Cheese is one of the world's most popular food products, providing a rich source of calcium, a mineral that can help boost energy levels. The health aspect of cheese focuses mainly on the role played by this specific mineral, but also on the specific role played by other components such as proteins, bioactives, peptides and Sphingolipids.

5.4 Cheese technology:

Cheese-making can be seen as an agglomeration phenomenon corresponding to the syneresis linked to the flow phenomenon, i.e. an agglomerate of milk protein elements, mainly casein, more or less modified and trapping the other elements. This agglomeration phenomenon is linked to the

flow of the liquid phase, made up of water, milk and soluble elements trapped in pores and then released.

The aim of cheese-making technology is to preserve milk and defer consumption over time. Product protection is mainly achieved by controlled lactic acidification and partial dehydration in the first two stages of production. The third stage, called refining, is the most common and corresponds to the transformation of substrates previously separated during draining by enzymatic and microbial processes **(Ramet, 1993).**

5.4.1 Milk preparation:

Ideally, milk is taken directly from the milking parlour to the dairy, where it is checked and tested for purity and cleanliness. It can then be pasteurized, usually at a temperature of 37°C for 15 second. The milk is then transferred to a vat and heated until it reaches the level of acidity required for the type of cheese to be made **(Harbutt., 2010).**

5.4.2 Coagulation:

The coagulation of milk corresponds to all the physico-chemical modifications taking place at the level of the casein micelles, resulting in the formation of a gel or, more specifically, a coagulum which traps the soluble elements of the milk. This is the most important stage in making a successful cheese **(Eck, 1997).**

5.4.3 Draining:

The gel state is physically unstable. The dispersing phase spontaneously separates from the coagulum in the form of whey. This separation is accompanied by the separation of the various original milk components: most of the water, lactose, and a small proportion of the fats and proteins are eliminated by the whey. Most of the proteins and fats remain in the coagulum, and the dry extract of the coagulum gradually increases as the serum is eliminated. Acidification of the milk before and after coagulation eliminates the mineral salts initially attached to the micelles. The level of residual protein mineralization determines the degree of coagulation of the coagulum, its ability to drain and the final dry extract of the cheese **(Ramet, 1993).**

5.4.4 Salting and molding:

Salting can be carried out by spraying and dry rubbing the surface of the cheeses, by immersion in brines, and for certain cheese products by addition to the curd mass before moulding.

EXPERIMENTAL PART

MATERIAL AND METHODS

Material and methods:

1 Chemicals

The compounds Folin–Ciocalteu phenol reagent, 1,1-diphenyl-picrylhydrazyl (DPPH), sodium carbonate, gallic acid, sulfuric acid, sodium phosphate, ammonium molybdate, porcine pancreatic α-amylase, trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid), ferrous chloride, ferrozine were purchased from Sigma–Aldrich (Germany), DNS and ABTS . All solvents used were of analytical grade and purchased from Prolabo (France).

2 Plant material:

The plant material used in this study corresponds to olive pomace (Ajerradj variety)(Figure10) . This olive by-product was collected from oil mill located at Barbacha (Béjaia) in December 2024. The sample was oven-dried at 50 °C for 3 days, then ground to a fine powder using an electric grinder. The grinding was followed by sieving (500 µm granulometry) using a sieve, and the powder was stored in a refrigerator at 4 °C.

Figure8: Photography of olive pomace powder.

3 Ultrasound-assisted extraction:

Extraction of phenolic compounds from olive pomace was carried out using an ultrasonic bath. A quantity of 1g of olive pomace powder was placed in a test tube with 20ml of extraction solvent and then introduced into the ultrasonic bath. After extraction, the content of the test tube was filtered and centrifuged for 15min at 3000rpm, then collected the supernatant.

4 Determination of total phenolic compounds:

Content of total phenolic compounds (TPC) was determined by the Folin-Ciocalteu method reported by Haddadi-Guemghar et al. (2014). A volume of 1 ml of Folin-Ciocalteu reagent (diluted 10 times) was mixed with 100µl of the extract. After 5 min, 1 ml of aqueous sodium carbonate solution was added. The mixture was kept for 30 min at room temperature then Absorbance was measured at 750 nm. Ethanol solution of gallic acid was used as standard.

5 Preliminary study:

A preliminary study for the selection of factors and their levels was carried out. In this study, the effects of three influential extraction parameters; solvent concentration, extraction time and temperature were systematically investigated separately as a single factor for setting up the optimum extraction conditions to obtain the maximum yield of phenolic compounds from olive pomace powder.

5.1 Optimization of extraction solvent concentration:

Extraction was performed with 20 ml of solvent for 1 g of olive pomace powder at different concentrations of aqueous ethanol 0%, 20%, 40%, 60% and 80% (v/v), which are placed in an ultrasonic bath at temperature of 40°C for 20 min. After each extraction, the mixture was filtered with filter paper and centrifuged. The supernatants obtained were covered and stored until analysis.

5.2 Optimization of extraction time:

A quantity of 1g of olive pomace powder was mixed with 20ml of 60% ethanol in test tubes which were placed in an ultrasonic bath set at 40°C for 10 min, 20 min, 40 min, 60 min and 90 min. After each extraction, the mixture was filtered and centrifuged at 3000rpm for 15min. The supernatants obtained were covered and stored until analysis.

5.3 Optimization of extraction temperature:

A quantity of 1g of olive pomace powder is extracted with 20 ml of 60% ethanol at different temperatures (20°C, 40°C, 60°C and 90°C). After 20min, the mixtures are centrifuged at 3000 rpm for 15 min, the supernatants were recovered and filtered.

5.4 Experimental design:

The analysis of experimental designs involves the use of reduced centered variables to designate the different levels of the factors. The variables in the experimental design are centered on 0, and are reduced to the interval [-1, +1]. The use of reduced centered variables allows for a better comparison, as differences in domain between factors are eliminated.

The table II shows the parameter levels chosen from the preliminary study to establish the experimental design.

Table II: Selected parameter levels.

	-1	**0**	**+1**
Time (min)	20	40	60
Temperature (%C)	20	40	60
Concentration (%)	40	60	80

The tableIV shows the experimental design obtained by the software using the levels of the independent variables in table II and the CCD design.

Table II: Experimental matrix.

configuration	**Ethanol concentration**	**Extraction time**	**Extraction temperature**
00-	60	40	20
+-+	80	20	60
00+	60	40	60
---	40	20	20
000	60	40	40
++-	80	60	20

000	60	40	40
--+	40	20	60
-++	40	60	60
000	60	40	40
-+-	40	60	20
+--	80	20	20
000	60	40	40
0-0	60	20	40
000	60	40	40
+0+	80	60	60
-00	40	40	40
0+0	60	60	40
000	60	40	40
+00	80	40	40

6 Preparation of dry extracts:

Extracts obtained under ideal conditions were placed in petri dishes, then dried in an oven at 50°C for 24 hours.

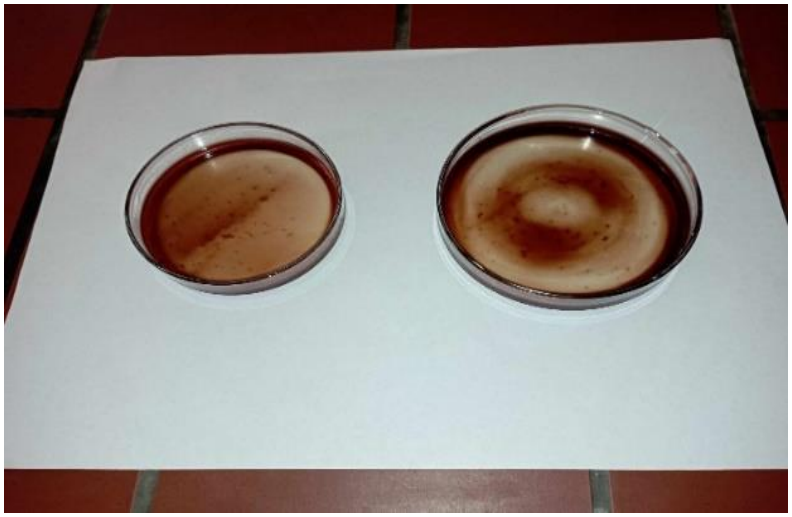


Figure9: Photograph of extract after drying.

6.1 Evaluation of antioxidant activity

The antioxidant activity of the extracts was estimated by the DPPH method, according to the procedure described by Haddadi-Guemghar et al. (2014). An aliquot of 1.5 mL of sample solution at different concentrations (20-100µg/mL) was mixed with 1.5 mL of ethanolic solution of DPPH (0.2 mM). The reaction mixture was incubated for 30 min in the dark at room temperature. The absorbance of the resulting solution was measured at 517 nm with a spectrophotometer. Ethanol instead of sample solution was used as a control. DPPH scavenging capacity of the tested samples was measured as the percentage inhibition and calculated using the following formula:

$$\textbf{Antioxidant activity (\%)} = \frac{Ac-As}{Ac} \times 100$$

Ac: absorbance of control.

***As*:** absorbance of sample

6.2 Anti-diabetic activity:

To assess the anti-diabetic activity of olive pomace extract, the inhibitory potential towards α-amylase was checked in vitro and compared with the therapeutic drug acarbose using a method reported by ALI (2020) .In test tubes, 500 µL of olive pomace extract at different concentrations (200-1000 µg/mL) was added to 500 µL of α-amylase solution (0.5 mg/mL in 0.02 M sodium phosphate buffer pH 6.9) This solution was pre-incubated at 25°C for 10 minutes. A volume of 500 µL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added and incubated at 25°C for 10 minutes. After adding 1000 µL of dinitrosalicylic acid (DNS) reagent, the tubes were incubated in boiling water for 5 minutes and then cooled to room temperature. The reaction mixture was diluted with 2 ml of distilled water and the absorbance was measured at 540 nm using a UV-Vis spectrophotometer. The control was prepared using the same procedure, replacing the extract with distilled water, while the activity of the standard was tested by replacing the extract with acarbose at different concentrations (200-1000 µg/mL). The α-amylase inhibitory activity was calculated as percentage inhibition using the following equation:

Anti-diabetic activity (%) = (AC -AE)/ (AC ×100)

AC: absorbance of control. **AE**: absorbance of extract / Acarbose

6.3 Anti-inflammatory activity:

Anti-inflammatory activity was assessed by the protein denaturation assay using the method reported by kar et al **(2012).**

A volume of 1ml of extract at different concentrations (200-1000µg/mL) was added to 4.5mL of BSA (Bovine Serum Albumin). The samples were incubated in a water bath at 37°C for 20 min, then at 57°C for 3 min. After cooling, 2.5 ml of phosphate buffer solution at pH 6.4 was added. Absorbance was measured at 660 nm. Indomethacin was used as the standard.

The percentage inhibition of protein denaturation was calculated as follows:

$$\textbf{Anti-inflammatory activity (\%)} = \frac{Ac - As}{Ac} \times 100$$

Ac: absorbance of control.

***As*:** absorbance of sample.

7 Cheese formulation and analyses

7.1 Fresh cheese production

Cheeses were produced according to the slightly modified method described by Ferreira et al. (2024). A volume of 4 L of pasteurized whole milk, bought in a local supermarket, was heated to 34 °C and 0.4g of mesophilic lactic ferment was added. Afterwards, when the milk reached 39 °C, 1 mL of rennet was added. The mixture was homogenised by continuously mixing with a glass stirrer, covered with a cloth, and left to rest for 30 min. Subsequently, the product obtained was strained to remove the serum and placed in a mould. Finally, it was placed in the refrigerator until the next day. Four cheeses were produced with addition of different quantities of olive pomace powder

Cheese A: control (fresh cheese with no added olive pomace powder) 0%

Cheese B: 250g fresh cheese with 0.5g olive pomace powder 0.2%

Cheese C: 250g fresh cheese with 1g olive-pomace powder 0.4%

Cheese D: 250g fresh cheese with 2g olive pomace powder 0.8%

7.2 Physicochemical analyses

7.2.1 pH measurement:

A quantity of 10g of fresh cheese homogenized in 90 ml of distilled water was used to measure pH using a pH meter previously calibrated with buffer solutions. **(Wehr, 2004).**

7.2.2 Determination of moisture content:

Moisture content is determined using an infrared desiccator, which emits infrared radiation to evaporate the water in the sample. Sample weight is checked using an integrated balance. A washed and dried capsule, containing 1gram of the sample to be analyzed, is placed in an infrared desiccator equipped with an integrated precision balance. The desiccator temperature varies according to the humidity of the sample. Results are displayed in percent on the desiccator screen.

7.2.3 Determination of fat content:

Fat content was determined using the Gerber acid-butyrometer method. This method is based on the dissolution of the fat to be determined by sulfuric acid under the influence of centrifugal force, and the addition of a small quantity of isoamyl alcohol. The fat separates into a clear layer, and the graduations of the butyrometer reveal the fat content **(ISO 488, 19.).**

3 gram quantity of fresh cheese is introduced into the butyrometer belly, to which sulfuric acid is added through the stem opening, until the acid level exceeds the cup. The butyrometer is placed in the Marie bath at 65°C until the cheese cracks. After two hours, the butyrometer is removed from the Marie bath. A volume of 1mL of iso-amyl alcohol is added to the sulfuric acid (H2SO4) and diluted to line 50 on the scale. Moderate agitation of the butyrometer is applied, followed by centrifugation at 1200 rpm for 5 min. Fat content is expressed in g /100 g of cheese and given by direct reading on the butyrometer.

7.3 Sensory analysis:

To better appreciate the organoleptic quality of the cheese, an organoleptic test was carried out using an expert jury for a sensory and hedonic analysis in the sensory analysis laboratory at the University of Bejaia. The questionnaire for this test is shown in Appendix.

- Statistical data were processed using XL-STAT software.

8 Statistical analysis:

A descriptive analysis of the results was carried out using Microsoft office Excel 2016 software, in order to determine the means and standard deviation.

F-test was used to assess the significance of the mathematical model. Means were compared for the significant model using the Tukey's test with a 5% level of significance in JMP Pro 17 version. Response surface methodology (RSM) was used to determine the optimal conditions for extraction. RSM was performed using the DESIGN EXPERT software (Version 8.0.1. Stat-Ease, Inc. Minneapolis, MN, USA)

RESULTS AND DISCUSSION

Results and discussion

1 Preliminary study:

In order to build the experimental design, preliminary studies are used to ascertain the experimental range for each independent variable.

1.1 The effect of solvent concentration on extraction:

Different solvent concentrations (ethanol 0%, 20%, 40%, 60% and 80%) were used to study the influence of solvent concentration on TPC extraction when the other extraction conditions were defined as follows: extraction time 20min and temperature 40°C. The results obtained for the different ethanol concentrations are summarized in Table III. According to the results obtained, methanol 60% gave significantly the best TPC yield. The ethanol range 40% and 80% was selected as the experimental range in the experimental design.

Table IIIV: Effect of ethanol concentration on phenolic compound extraction

Ethanol concentration	0%	20%	40%	60%	80%
TPC (mgEAG/100gDP)	363.76±29.11c	435.66±27.72b	376.33±20.14c	645.16±16.23a	340.73±26.55c

Results are presented as means ± standard deviation. Different letters indicate a significant difference according to ANOVA and Turkey's test ($p < 0.05$).

1.2 The effect of extraction time:

The results of the determination of phenolic compounds in the extracts analyzed are presented in the table below, different extraction durations : 10, 20, 40, 60, and 90 minutes had an impact on TPC recovery when the temperature and solvent concentration were adjusted at 40°C and 60%, respectively.. The amount of phenolic compound extracted can be influenced by increasing the extraction time.

One crucial factor in maximizing phenolic compound extraction and minimizing process costs is extraction time. One of the most crucial parameters to monitor is the duration of contact between the solid and liquid phases since it is closely related to the extraction's kinetics. Understanding this last one will enable the extraction to be stopped when the desired result is obtained.

Extraction times mark a minimum value of 100.04mg EAG /100gDP at 10min then it marks a progressive increase of TC contents up to a maximum value of 1003. 30mg/100g at 40min.

TableV: Influence of the time on the phenolic content of olive pomace.

Time	10min	20min	40min	60min	90min
TPC (mg/100g)	520.16± 48.93c	966.33± 56.71ab	1003.03± 130.29a	680.03± 129.98bc	645.13± 153.67c

Results are presented as means ± standard deviation. Different letters indicate a significant difference according to ANOVA and Turkey's test ($p < 0.05$).

1.3 The effect of extraction temperature:

The results show that TPC extraction was significantly influenced by extraction temperature ($p<0.05$). Extraction increased with increasing temperature. Results for TPC content of olive pomace extracts ranged from 524.33mg /100 g to 1837. 40 mg /100 g. A significant increase in TPC was observed over the extraction temperature range (20-90C°), with phenolic content reaching a maximum of around 1837. 40mg /100g DP at 20C°.

Table IV: Influence of the temperature on the phenolic content of olive pomace

Temperature	20C°	40C°	60C°	90C°
TPC(mg/100g)	1837.40± 101.11a	558.29± 53.88c	524.33± 15.42c	1122.43± 50.87b

Results are presented as means ± standard deviation. Different letters indicate a significant difference according to ANOVA and Turkey's test ($p < 0.05$).

1.4 Experimental design:

The values for the experimental design come from the 20 tests that are shown in the table below. The CPT content varied between 143.314mgEAG/100gDP and 1027.645 mg /100 g of dry powder, according to the data, indicating that temperature, time, and solvent concentration all affect TPC extraction. The investigation done **by Ballard et al. (2010)** validated the impact of these parameters.

Table V: Experimental design test results.

	Ethanol concentration	Extraction time	Extraction temperature	TPC
1	60	40	20	717.099
2	80	20	60	249.408
3	60	40	60	936.301
4	40	20	20	143.314
5	60	40	40	1001.899
6	80	60	20	668.61
7	60	40	40	924.766
8	40	20	60	565.2
9	40	60	60	1027.645
10	60	40	40	887.364
11	40	60	20	311.27
12	80	20	20	500.654
13	60	40	40	889.578
14	60	20	40	543.773
15	60	40	40	978.28
16	80	60	60	697.401
17	40	40	40	653.471
18	60	60	40	929.094
19	60	40	40	1008.308
20	80	40	40	725.49

1.4.1 Model fitting:

The analysis of variance (ANOVA) results for the quadratic polynomial model fitted for TPC performance are presented in the table below. The F-test suggests that the model has a very high F-value and a very low p-value ($p < 0.0001$), indicating that the model was highly significant. The p-value for lack of fit was greater than 0.05, indicating that it was not statistically significant compared with the pure error and that the model equation was suitable for predicting TPC performance for any combination of variable values.

Table VI: Experimental design test results.

Source	Sum of **Squares**	**df**	Mean **Square**	F **Value**	P value **Prob > F**	
Model	1,315E+006	9	1,461E+005	48,12	< 0.0001	significant
A-Ethanol fraction	*1978,80*	*1*	*1978,80*	*0,65*	*0,4382*	
B-Extraction time	*2,662E+005*	*1*	*2,662E+005*	*87,72*	*< 0.0001*	
C-Extraction temperature	*1,288E+005*	*1*	*1,288E+005*	*42,44*	*< 0.0001*	
AB	*26,10*	*1*	*26,10*	*8,599E-003*	*0,9279*	
AC	*2,314E+005*	*1*	*2,314E+005*	*76,25*	*< 0.0001*	
BC	*41257,72*	*1*	*41257,72*	*13,59*	*0,0042*	
A^2	*1,090E+005*	*1*	*1,090E+005*	*35,90*	*0,0001*	
B^2	*63617,73*	*1*	*63617,73*	*20,96*	*0,0010*	
C^2	*10512,34*	*1*	*10512,34*	*3,46*	*0,0924*	
Residual	30351,39	10	3035,14			
Lack of Fit	*15260,10*	*5*	*3052,02*	*1,01*	*0,4953*	*not significant*
Pure Error	*15091,29*	*5*	*3018,26*			
Cor Total	1,345E+006	19				

The model's goodness of fit was evaluated using a number of descriptive statistical analyses, such as coefficient of determination (R^2), adjusted coefficient of determination ($AdjR^2$), predicted coefficient of determination (Pred. R2), appropriate precision (Adeq Precision), and coefficient of variation (CV). The sample variation was statistically significant at 97,74%, according to the R2 value of 0,9774, which also indicated that the model could only explain roughly 2.26% of the total variance. Stated otherwise, a coefficient of determination in the neighborhood of 1 denotes a strong correlation between the observed and predicted data.

The model's significance was also satisfactory confirmed by TPC's Adj R^2 and Pred R^2. There was a 0.0284 difference between the two indices, indicating a reasonably close link between Pred R^2 and $AdjR^2$. CPT's Adj R^2 and Pred R2 were also satisfactory in confirming the importance of the model. The relationship between Pred R^2 and $AdjR^2$ was reasonably close, with a difference of 0.0284 between the two parameters, less than 0.10. The coefficient of variation (CV) represents the level of dispersion in the data. As a general rule, a low CV value leads to better reproducibility while a high CV value (superior to 10%) indicates high variance of the mean value and the inability to develop an adequate response model.

Table VII: Descriptive parameters for model fitting.

Std. Dev	55,09	**R-Squared**	0,9774
Mean	717,95	**Adj R-Squared**	0,9571
C.V. %	7,67	**Pred R-Squared**	0,9287
PRESS	95873,78	**Adeq Precision**	23,029

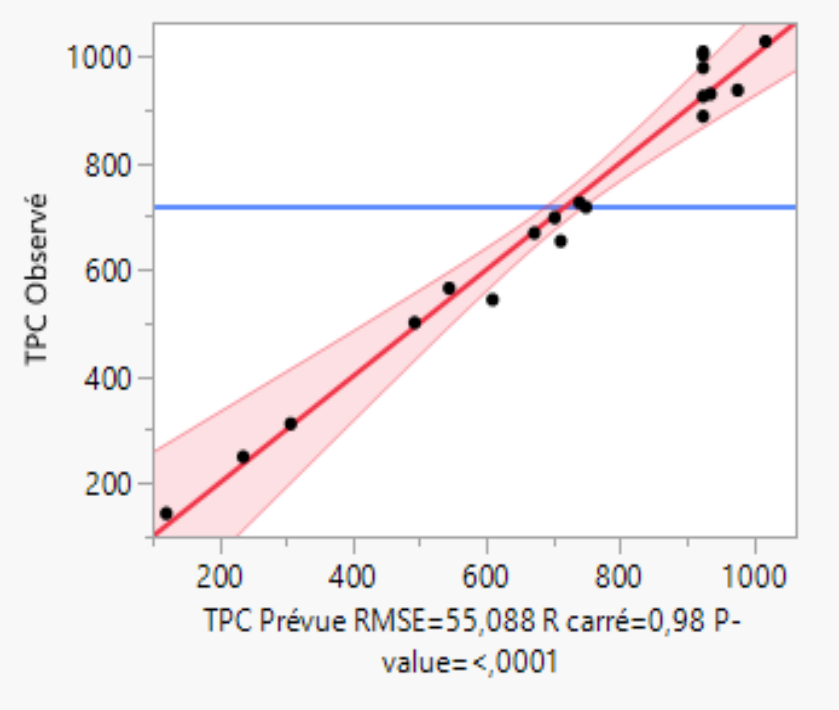


Figure 10: Observed values plotted against predicted values

1.4.2 Variable significance:

The significance of each coefficient was determined using the p-value. This value is used as a tool to assess the significance of each coefficient and the strength of interactions between each independent variable. A p-value of less than 0.05 indicates that the model terms are significant. In this case, the main factors affecting TPC extraction were extraction time and extraction temperature followed by solvent concentration. After neglecting all non-significant terms (p>0.05), the fitted quadratic model for TPC in coded variables are given in the following equation:

TPC=+924, 43 +163, 17*B+113, 50*C-170, 09*AC+71, 81*BC-199, 05*A^2-152, 10*B^2

1.4.3 Response surface analysis:

The 3D response surface is the three-dimensional graphical representation used to determine the individual and cumulative effect of mutual interaction between variables. The response surface analyzes the geometric nature of the surface, and the maxima and minima of the response. It provides a method for visualizing the relationship between responses and experimental levels of each variable, and the type of interactions between two test variables.

Figure13 is a response surface plot showing that by increasing ethanol concentration and extending time, extraction yield (CPT) increases progressively to reach its maximum 980mg/100g at time 45min and ethanol 60%. This maximum CPT decreases slightly with increasing sonication time and ethanol concentration.

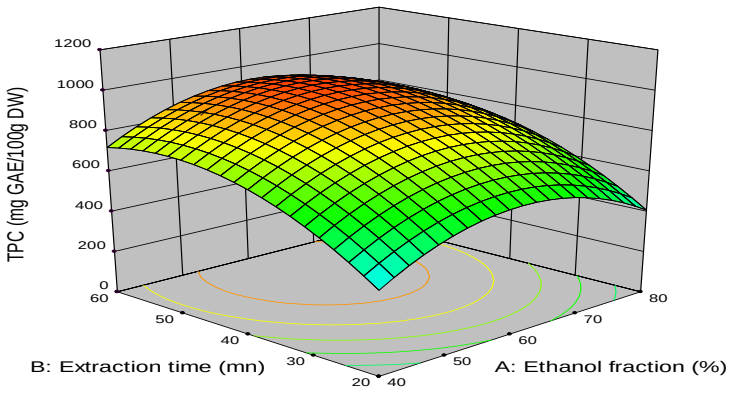


Figure 11:3-D curve showing the interaction effect of ethanol concentration parameter and extraction time on TPC yield.

The figure14 is a response surface plot showing the interaction between ethanol concentration and extraction temperature on CPT yield, keeping sonication time constant. By increasing ethanol concentration and extraction temperature in the chosen experimental range. The extraction yield increases up to around 1000mgGAE/100gDW with ethanol concentration 55% and at 60C° afterwards the yield decreases slightly with increasing ethanol concentration.

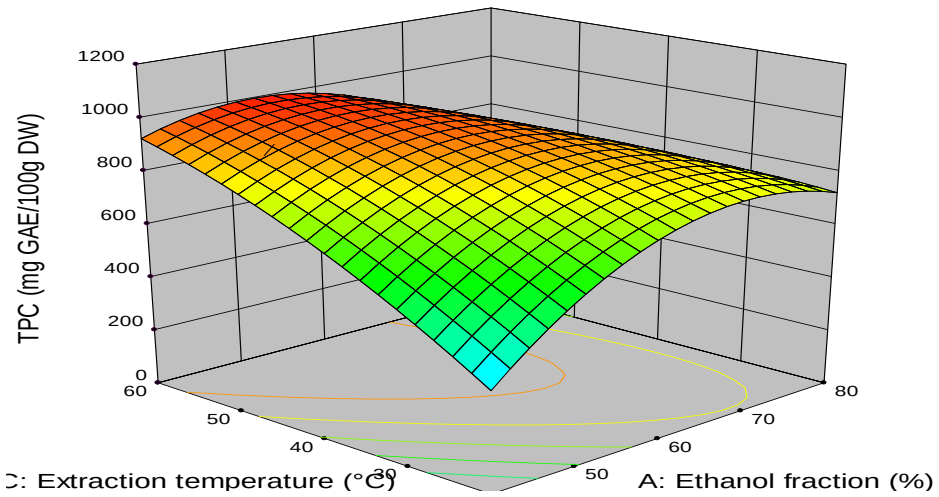


Figure 12:3-D curve showing the interaction effect of ethanol concentration parameter and extraction temperature on TPC yield

The figure15 shows the effect of temperature and extraction time on CPT yield. CPT yield increases with increasing extraction time. The best extraction of CPT is 1095mgGAE/100g DW obtained at55min and temperature 55°C.

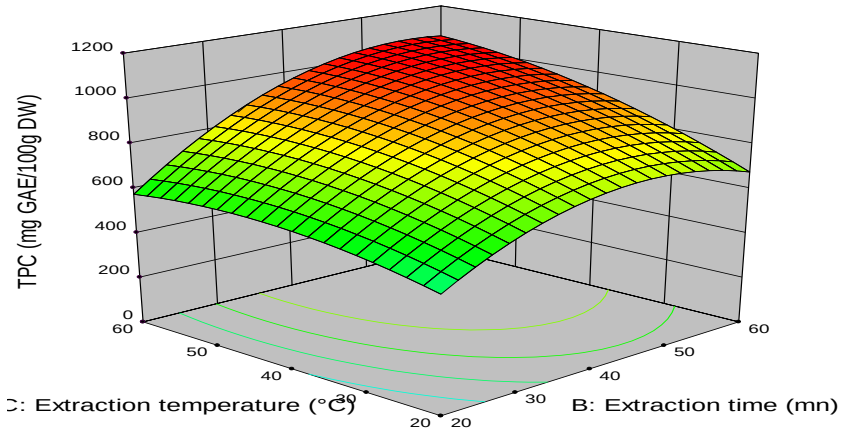


Figure 13: 3-D curve showing the interaction effect of time extraction and extraction temperature on TPC yield.

1.5 Optimal extraction conditions:

Optimization using the desirability function (0.99) revealed the following as the ideal circumstances for TPC ultrasound assited extraction from olive pomace (Figure 16° :

- Ethanol 52,06% as the extraction solvent.

- 55, 49 minutes as the extraction time.

- 60°C as the extraction temperature.

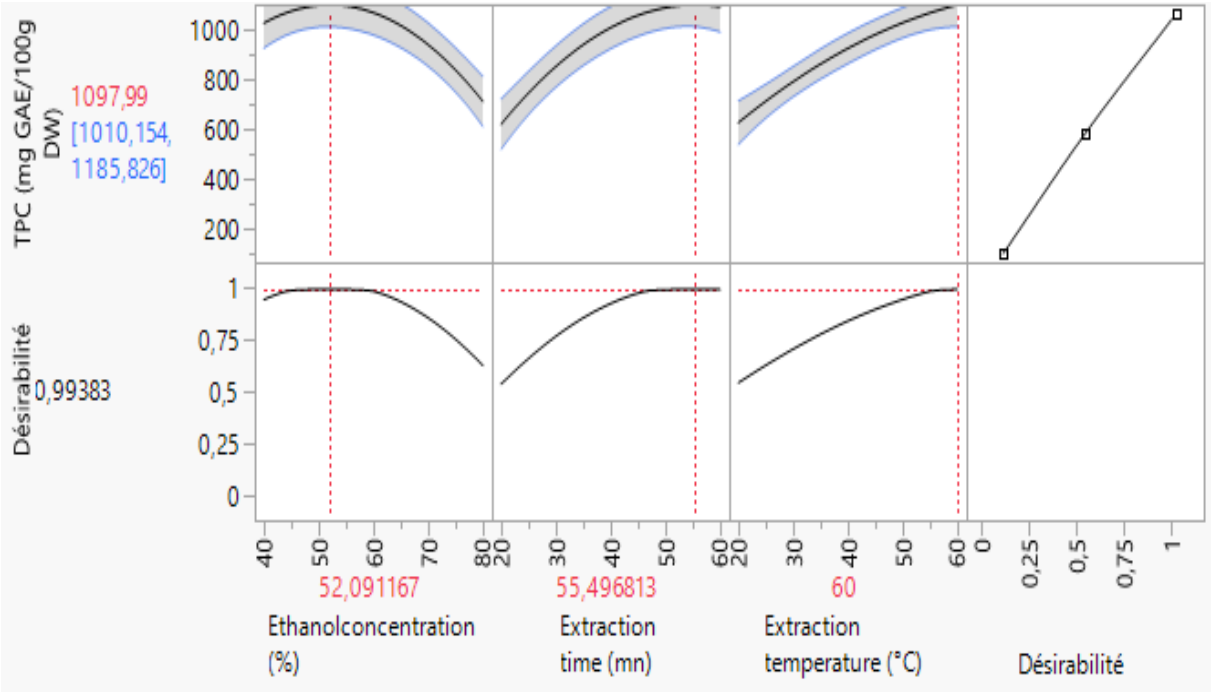


Figure 14: Profiles for predicted values and desirability function**.**

1.6 Model validation:

The optimized conditions obtained by the response surface methodology were used to validate the model predicted for the extraction of phenolic compounds from olive pomace. The table verifies the validity and applicability of the mathematical model by demonstrating that the experimental values are fairly near to the expected values. The predicted value and the experimental value do not differ significantly.

Table VIII: Optimum conditions for extracting TPC from olive pomace expressed in mg EAG/100g DP

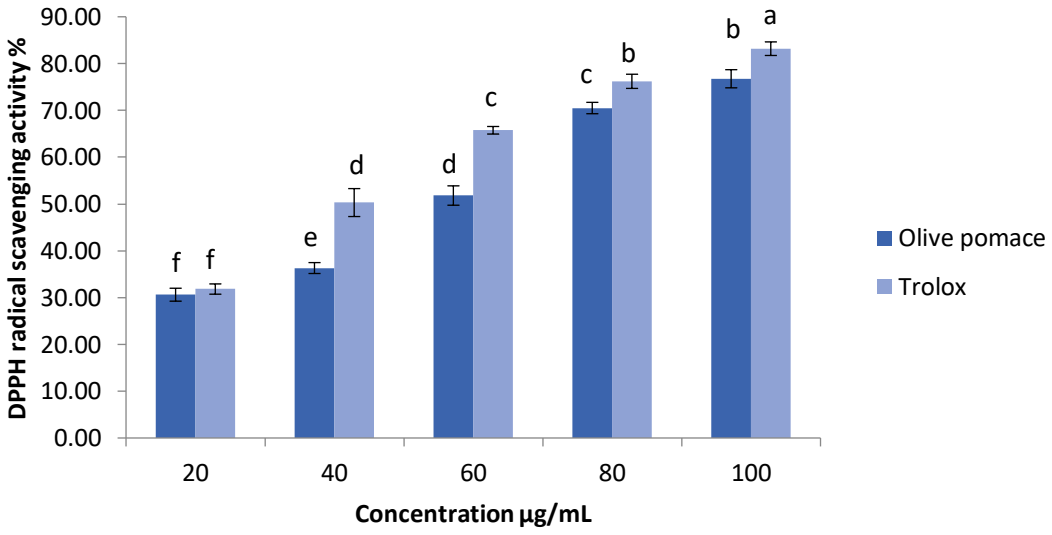
Settings	Ethanol concentration (%)	Temperature C°	Extraction time	predicted value	experimental value
TPC(mgEAG/100 gDP)	52,09%	60C°	55,49min	1097,99± 87,73	1106± 56,67

1.7 Antioxidant activity

Figure below shows that olive pomace has a higher relative free radical scavenging activity than trolox at all the concentrations tested.

The relative free radical scavenging activity of olive pomace increases with concentration.

The relative free radical scavenging activity of trolox also increases with concentration, but at a slower rate than olive pomace.The IC50 values of olive pomace and trolox were determined in a separate study. The IC50 value is the concentration of a compound required to inhibit free radical activity by 50%. The IC50 values for olive pomace and trolox were 42.19 µg/mL and 103.50 µg/mL, respectively. These results are consistent with the histogram results, which show that olive pomace has a higher relative anti-free radical activity than trolox.



The vertical bars represent the standard deviation. The different letters on the bars show that there is significant differences ($p \leq .05$). The values denoted by the identical letters do not show significant differences ($p \leq .05$). The results are listed in descending order a>b> c > d> e > f

1.8 Antidiabetic activity:

The histogram compares the anti-diabetic activity of acarbose and olive pomace, expressed as a percentage. The histogram shows that no signifcative differences in anti-diabetic activity between olive pomace and acarbose at all concentrations tested. The relative anti-diabetic activity of olive pomace and acarbose increased with concentration. The IC50 values for olive pomace and acarbose were 12.18 µg/mL and 20.44 µg/mL, respectively. These results are consistent with the histogram results, which show that olive pomace has a higher relative Anti-diabetic activity than acarbose

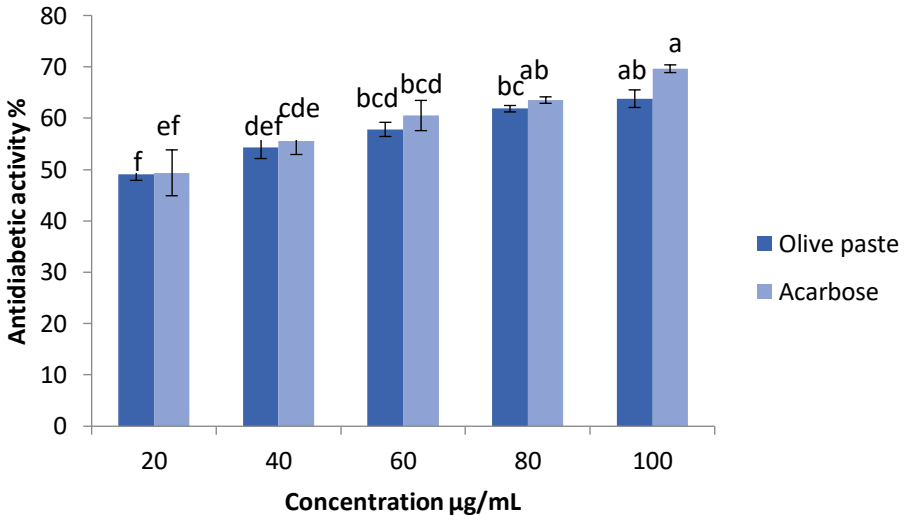


Figure15: Percentage of anti-diabetic activity of ethanolic olive pomace extract compared with acarbose.

The vertical bars represent the standard deviation. The different letters on the bars show that there is significant differences ($p \leq .05$). The values denoted by the identical letters do not show significant differences ($p \leq .05$). The results are listed in descending order a>b> c > d> e > f

1.9 Anti-inflammatory activity:

The histogram compares the anti-inflammatory activity of olive pomace and indomethacin, expressed as a percentage. Olive pomace has a significatively higher anti-inflammatory activity than indomethacin at all concentrations tested. The IC50 values for olive pomace and indomethacin were 823.56 µg/mL and 1026.81 µg/mL, respectively (Table X). These results are consistent with the histogram results, which show that olive pomace has a higher relative anti-inflammatory activity than indomethacin.

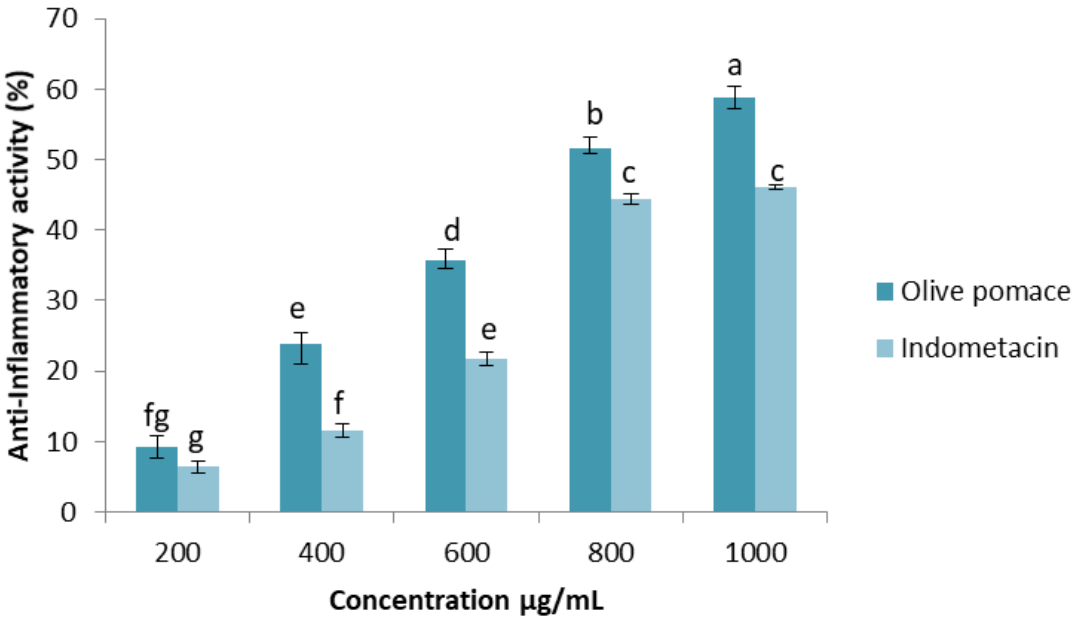


Figure 16*:* Percentage of anti-inflammatory activity of ethanolic olive pomace extract compared with acarbose**.**

The vertical bars represent the standard deviation. The different letters on the bars show that there is significant differences ($p \leq .05$). The values denoted by the identical letters do not show significant differences ($p \leq .05$). The results are listed in descending order a>b> c > d> e > f > g

Table IX: Biological activity of olive pomace expressed as IC50

Biological activity	**Tested compounds**	**IC50**	N'nabinty Sylla et al **(2021).**
Antioxidant activity	Olive pomace extract	103.50±6.68	200 ± 0.15
	Trolox	42.19±0.85	106.29 ± 2.46
Anti-diabetic activity	Olive pomace extract	20.44±4.24	100.19 ± 3.50
	Acarbose	12.18±4.18	116.25 ± 2.04
Anti-inflammatory activity	Olive pomace extract	823.56 ± 11.29	_
	indomethacin	1.81 ± 5.52	_

2 Fresh cheese analyses

2.1 Physicochemical analysis of cheese

- **pH:**

The pH of fresh cheese (4.98) and cheese with olive pomace (4.99) did not change significantly in our investigation (Table XI), and these findings are consistent with standards (4.44±5.63). The results show that there is no acidification following the addition of olive pomace because the pH values are all quite near to one another and lie within the 4.44±5.63 range.

- **Moisture content**:

The results (Table XI) show that the moisture content is significantly similar in both cheeses, indicating that the addition of olive pomace does not affect the moisture content of the fresh cheese

- **Fat content:**

According to the data (Table XI), the fat content of both cheeses is close to 20%, with the olive pomace cheese having 18.6±1.33% fat and fresh cheese having 19.5% fat.

Table X: Results of physicochemical analyses of fresh cheese.

Parameter **Sample**	**pH**	**Moisture%**	**% Fat**
Fresh cheese	4.98± 0.23	41.95±0.98	19.5±1.34
Cheese with olive pomace	4.99±0.32	42.89± 1.56	18.6±1.33
standards	4,44 ± 5,63	35à60	20

2.2 Sensory analysis:

Before running the various tests on XLSTAT, an experimental design was created. Once the data from the expert juries had been reported to the software, the experimental design generation procedure was launched. For each of the categories: sensory analysis and hedonic analysis, an optimal experimental design was found, which validated the other tests on XLSTAT.

Product characterization

Product characterization enables us to identify the descriptors that best discriminate between products, and the characteristics that are important for sensory analysis.

2.2.1 Discriminating power by descriptor

This test is illustrated in figure below, and displays descriptors ordered from the one with the highest discriminating power to the one with the lowest.

The graph below shows the descriptors ordered from most to least discriminating on four fresh cheese samples. They show that each of texture, color, aroma, appearance and consistency are the most discriminating descriptors. In other words they differ between the cheese samples. On the other hand, the least discriminating descriptor is tartanability, it means that there is no difference in tartanability between the four fresh cheese samples according to the judges.

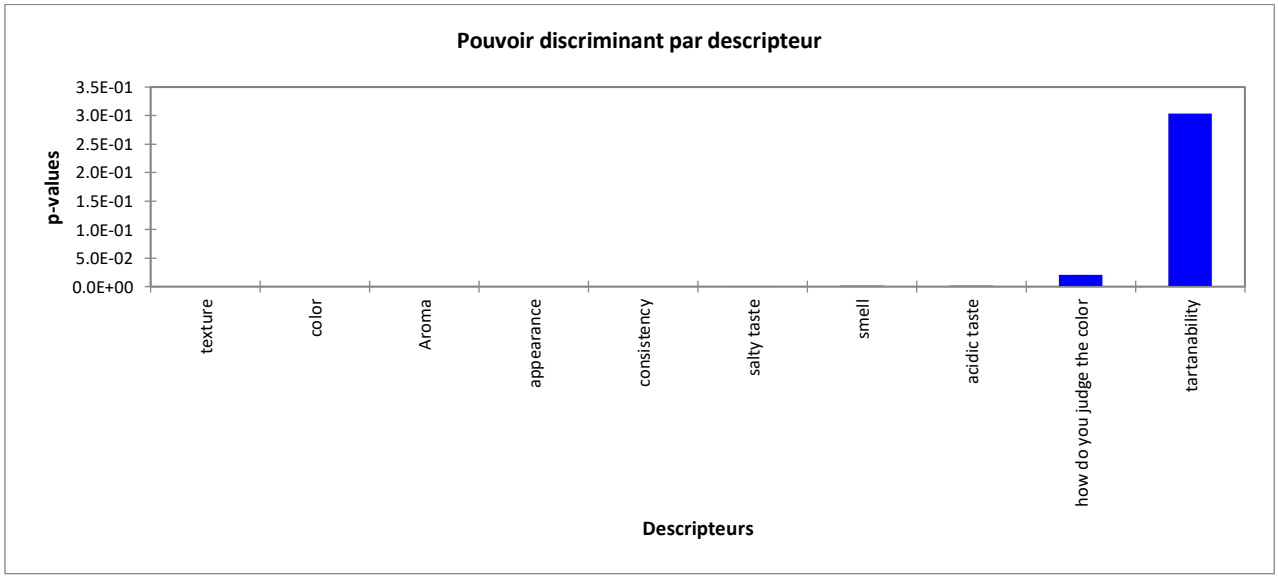


Figure 17: Discriminating power by descriptor

2.2.2 Model coefficients:

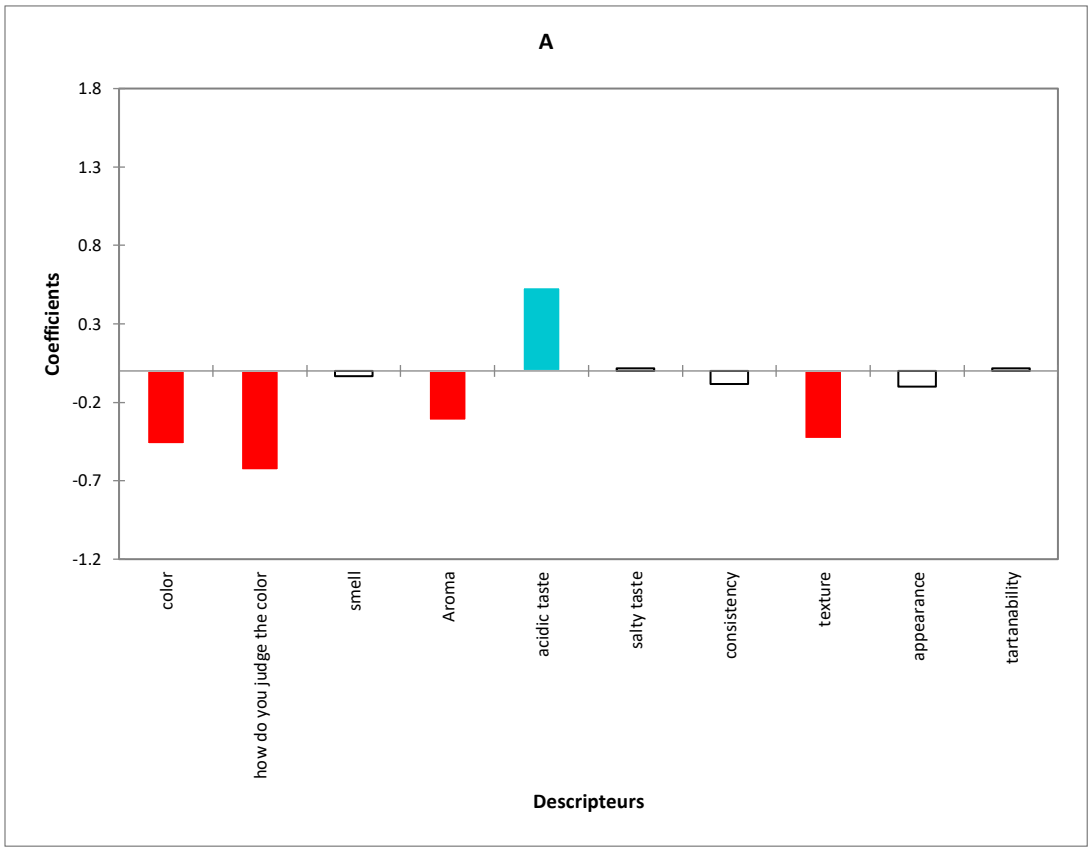
The coefficients of the selected model for each descriptor and product are shown in the figure below; the next picture summarizes what characterizes the product (cheese A, B, C, and D). Characteristics with significantly positive coefficients are indicated in blue, those with considerably negative coefficients in red, and those with non-significant coefficients in white. In the figue below the bars are colored differently to indicate the importance and direction of the impact of the descriptors. Red bars indicate significant negative coefficients, while light blue bars indicate significant positive coefficients and white indicates characteristics whose coefficients are not significant

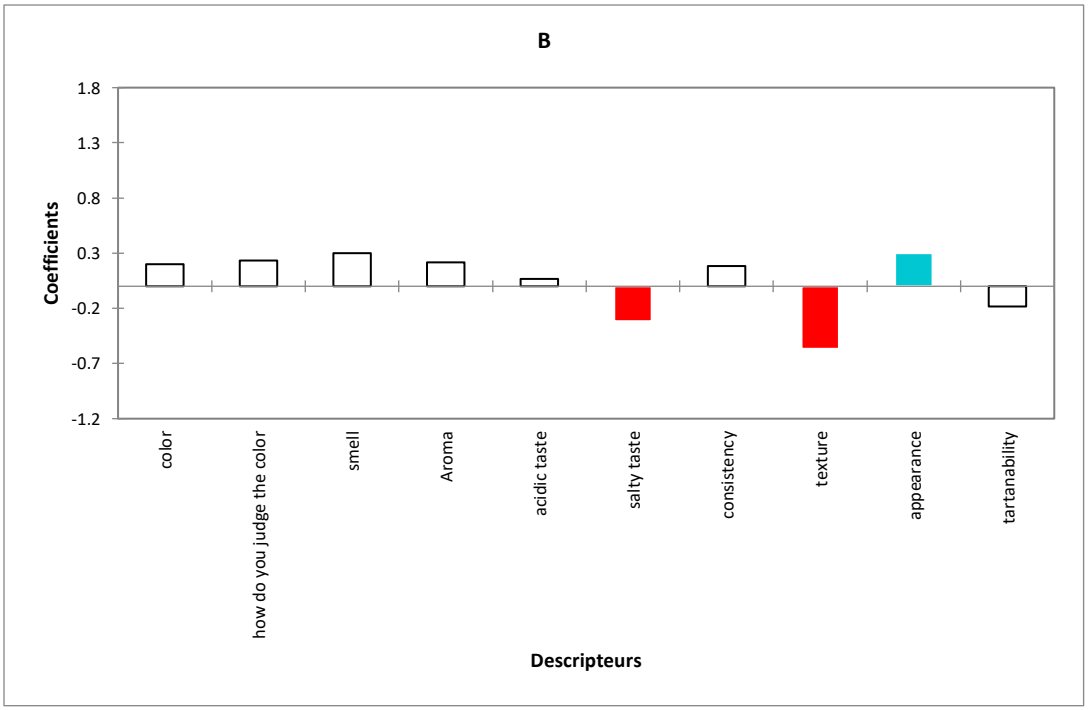
Sample A: Acidic taste has a light blue coefficient, suggesting a significant positive impact on the dependent variable. This means that participants seem to prefer an acidic taste. for Odor, Consistency and Appearance These descriptors have coefficients very close to zero, indicating that they have no significant impact, on the other hand ,color , color judgment , aroma and texture have a red Coefficient, indicating a significant negative impact.

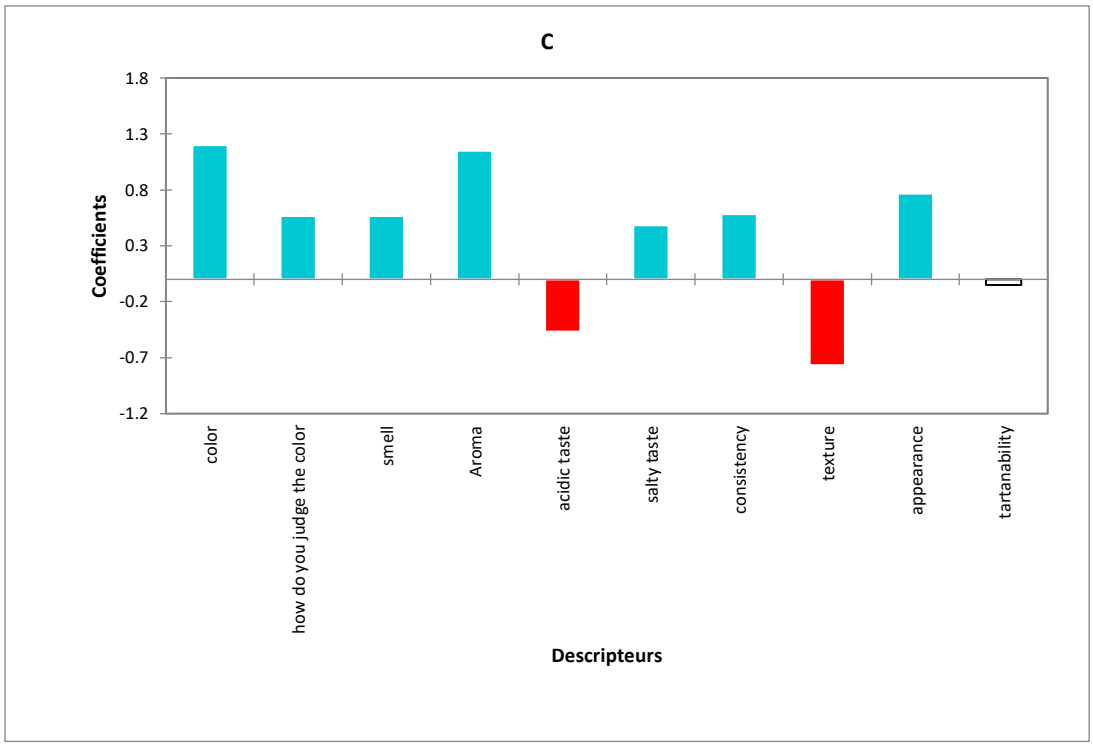
Sample B: the coefficient for descriptors such as appearance, consistency and color is positive, seeming to improve the dependent variable, while descriptors such as salty taste and texture have a significant negative impact, suggesting that products with these characteristics are rated less highly by participants.

Sample C: Color, aroma and appearance coefficients have significant positive impacts, while acid taste and texture coefficients have negative impacts

Sample D: color, odor, aroma, consistency, salty taste and appearance all have a significant positive impact, whereas acid taste and texture have a negative impact


A
1.8
1.3
0.8
0.3
-0.2
-0.7
-1.2
Coefficients
color
how do you judge the color
smell
Aroma
acidic taste
salty taste
consistency
texture
appearance
tartanability
Descripteurs


B
1.8
1.3
0.8
0.3
-0.2
-0.7
-1.2
Coefficients
color
how do you judge the color
smell
Aroma
acidic taste
salty taste
consistency
texture
appearance
tartanability
Descripteurs



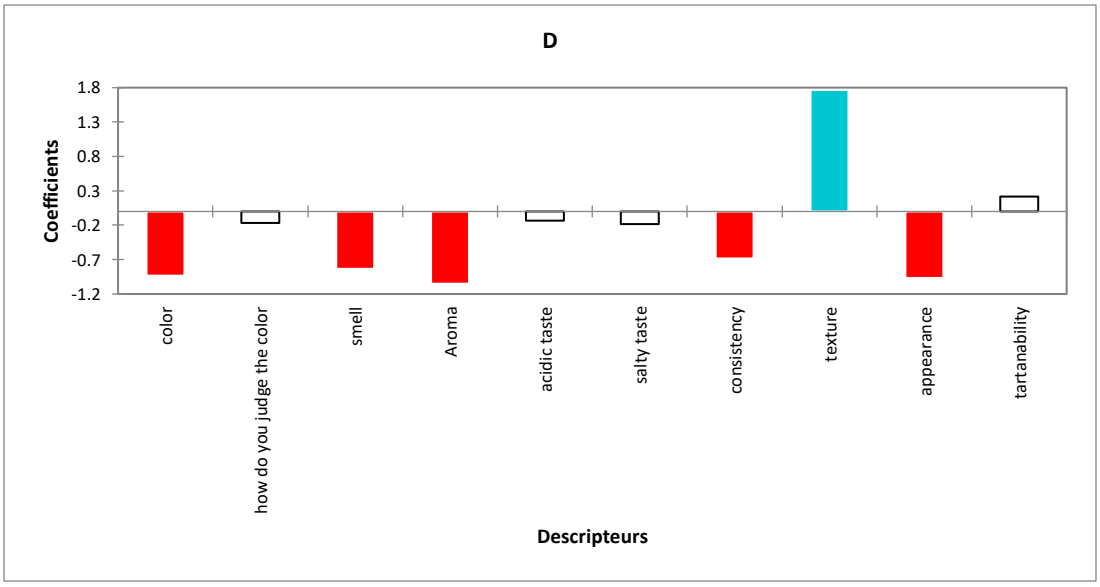


Figure18: Model coefficients for the four cheese samples

The bars are colored differently to indicate the importance and direction of the impact of the descriptors. Red bars indicate significant negative coefficients, while light blue bars indicate significant positive coefficients**.**

Sample A: Acidic taste has a light blue coefficient, suggesting a significant positive impact on the dependent variable. This means that participants seem to prefer an acidic taste. for Odor, Consistency and Appearance These descriptors have coefficients very close to zero, indicating that they have no significant impact, on the other hand ,color , color judgment , aroma and texture have a red Coefficient, indicating a significant negative impact.

Sample B: the coefficient for descriptors such as appearance, consistency and color is positive, seeming to improve the dependent variable, while descriptors such as salty taste and texture have a significant negative impact, suggesting that products with these characteristics are rated less highly by participants.

Sample C: Color, aroma and appearance coefficients have significant positive impacts, while acid taste and texture coefficients have negative impacts

Sample D: color, odor, aroma, consistency, salty taste and appearance all have a significant positive impact, whereas acid taste and texture have a negative impact

2.2.3 Adjusted averages by product:

The product-adjusted mean test is a powerful method for comparing products in sensory analysis, while controlling for confounding variables, thus providing more reliable and accurate results.

Sample A: Only the acidic taste is significantly positive, while aroma, color and texture have a significant negative effect, making the acidic taste the characteristic most appreciated by the judges.

Sample B: The appearance descriptor has a significant positive effect on the product, while the salty taste and texture have a significant effect and the rest of descriptors are not significant.

Sample C: aroma, consistency, appearance, color, smell and salty taste have a significantly positive effect on the product. Acid taste and texture have a significantly negative effect and are less appreciated in the sample c.

Sample D: The appearance descriptor has a significant positive effect on the product, while the salty taste and texture have a significant effect.

Table XI: Adjusted averages by product.

	Aroma	Consistency	Appearance	Color	Smell	how do you judge the color	salty taste	acidic taste	Tartanability	Texture
C	3,467	2,800	4,333	4,067	3,067	4,067	3,600	3,333	4,267	1,933
B	2,533	2,400	3,867	3,067	2,800	3,733	2,800	3,867	4,133	2,133
A	2,000	2,133	3,467	2,400	2,467	2,867	3,133	4,333	4,333	2,267
D	1,267	1,533	2,600	1,933	1,667	3,333	2,933	3,667	4,533	4,467

2.2.4 Preferred external mapping (PREFMAP):

The preference method links consumer preferences to the organoleptic characteristics of products. This approach is essential if marketing teams are to adjust products to consumer tastes. It enables objects and preference indications from expert judges to be visualized on a two- or three-dimensional graph, facilitating product adaptation.

2.2.5 Principal Component Analysis (PCA):

PCA can be seen as a projection method that projects observations from the p-dimensional space of p variables into a k-dimensional space, ($k < p$) such that a maximum of information is retained (information is measured here through the total variance of the scatterplot) on the first dimensions. Observations can be represented on a 2- or 3-dimensional graph, greatly facilitating interpretation. The figure below shows that the characteristics of the 4 samples are different

Sample A: is characterized by its strong acidic taste

Sample B: is characterized by its soft consistency, odor and good appearance

Sample C: is characterized by its well-appreciated color and salty taste

Sample D: is characterized by its soft texture and easy tartanability.

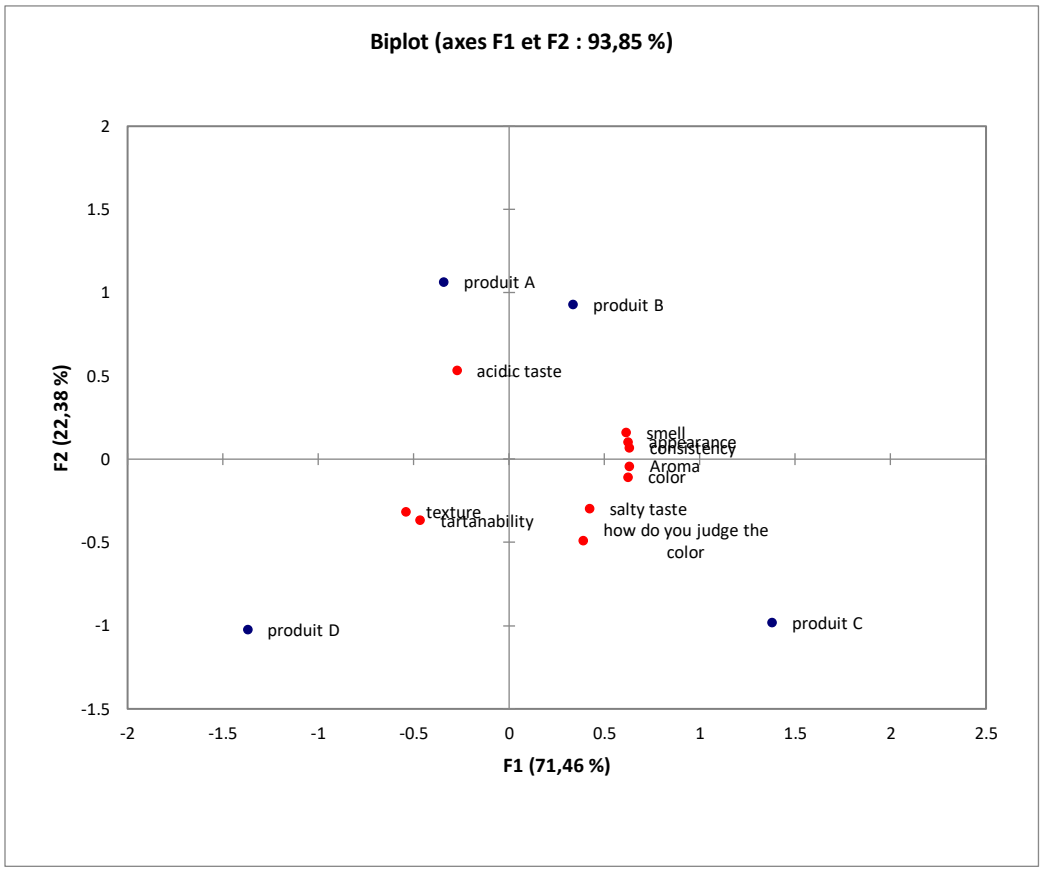


Figure19: Correlation between variables and factors**.**

2.2.6 Hierarchical ascending classification (HAC)

The HAC data analysis tool provides numerous tables and graphs. The class profile graph (made from preference data) allows for visual comparison of the averages of the many classes created. According to figure below three consumer classes were created from the preference scores:

Class 1: this class prefers sample C, then samples A and B AND finally sample d

.**Class 2 :** this class also prefers sample C then b then a and D last.

Class 3: unlike class A and B, this class prefers sample D, then A and B, and sample c last.

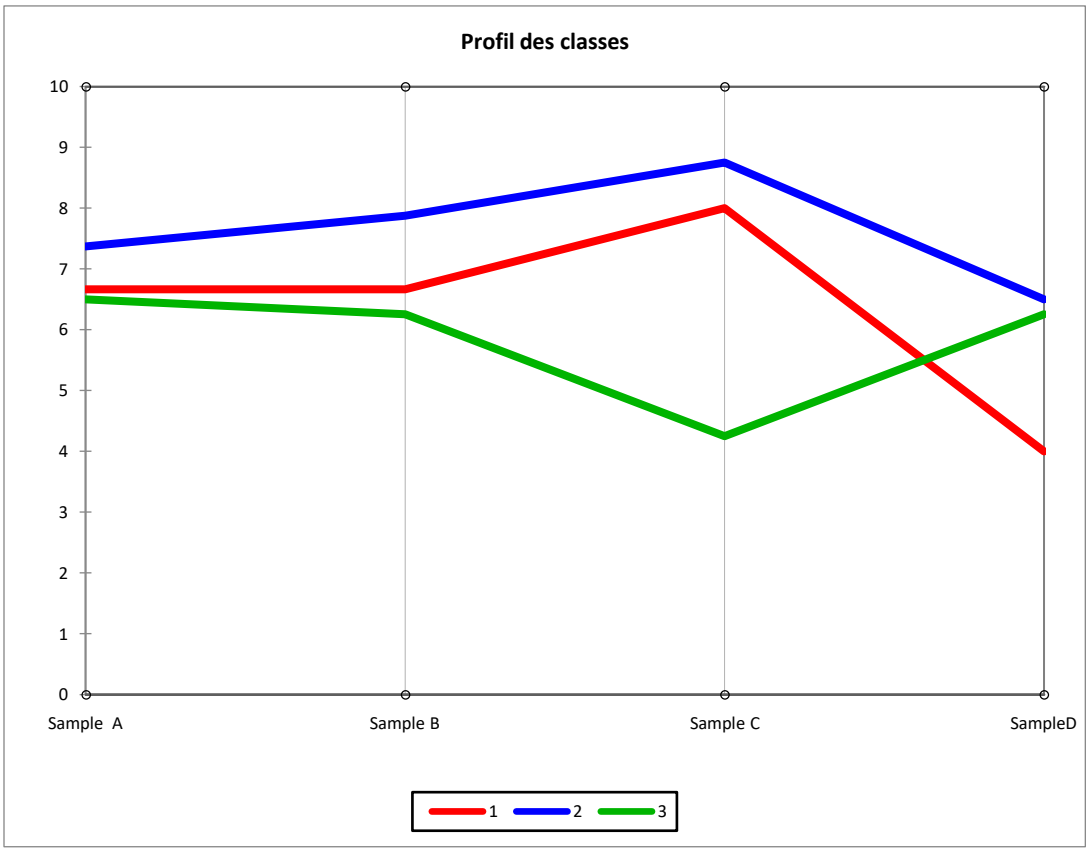


Figure 10: Profile of classes created.

2.2.7 PREFMAP preference mapping:

This test was carried out to find out the judges' preferences for our products.

The figure below shows that samples B and C are the most preferred and the first one is the most preferred, whereas samples A and D are not really preferred.

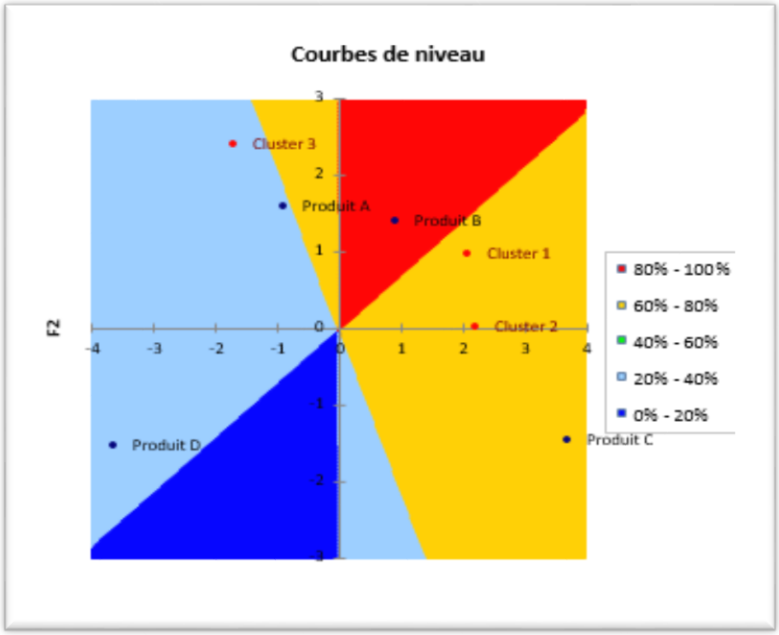


Figure 21: PREFMA preference map.

CONCLUSION

Conclusion

The aim of this study was to add value to olive pomace by extracting total phenolic compounds (TPCs) and incorporating this industrial waste into fresh cheese.

The ultrasound-assisted extraction strategy was developed to extract TPCs. Extraction parameters were optimized using a surface response methodology (SRM). The latter was successfully used to study the influence of ethanol concentration, temperature and extraction time on TPC yield. The optimum extraction conditions were: ethanol 52% extraction temperature 60°C and extraction time 55min.

Very interesting results were obtained when evaluating the antioxidant activity, anti-inflammatory and anti-diabetic effect of olive pomace extract, leading us to define this waste product as a functional food.

The incorporation of olive pomace at different concentrations (0.5, 1 and 2%) into fresh cheese produced a product whose physico-chemical characteristics were in line with cheese industry standards, and which was appreciated by both the expert jury and the general public. However, it should be emphasized that the fresh cheese with 2g of olive pomace was the most appreciated.

ANNEX

Annex

Questionnaire de l'analyse sensorielle du fromage frais

Sexe : H ou F Age : …… N° de poste : ….. Date : .. / .. / ….

Quatre échantillons du fromage frais enrichis codé A, B, C et D vous sont présentés, il vous est demandé d'évaluer différentes caractéristiques et attribuer une appréciation selon les codes donnés de 1 à 5.

NB : veuillez rincer la bouche à chaque dégustation d'un échantillon.

1) **La Couleur** :
   1. Blanc
   2. Blanc cassé
   3. Beige
   4. Jaune
   5. Jaune foncé

A	B	C	D

2) **Comment jugez-vous la couleur :**

1. n'est pas appréciée
2. peu appréciée
3 .moyennement appréciée
4. bien appréciée
5. très bien appréciée

A	B	C	D

3) Intensité de l'odeur (sans gouter):

1. Très faible
2. Faible
3. Moyenne
4. Forte
5. Très forte

A	B	C	D

4) Intensité de l'arôme (sensation en bouche):

1. Très faible
2. Faible
3. Moyenne
4. Forte
5. Très forte

A	B	C	D

5) Gout acide :

1. Très faible
2. Faible
3. Moyenne
4. Forte
5. Très forte

A	B	C	D

6) Gout salé :

1. Très faible
2. Faible
3. Moyenne
4. Forte
5. Très forte

A	B	C	D

7) Consistance :

1. Très molle
2. molle
3. Moyenne
4. ferme
5. Très ferme

A	B	C	D

8) Texture :

1. Très granuleuse
2. Granuleuse
3. Moyenne
4. Lisse
5. Très lisse

A	B	C	D

9) **Comment jugez-vous l'aspect ?**

1. n'est pas appréciée
2. peu appréciée
3 .moyennement appréciée
4. bien appréciée
5. très bien appréciée

A	B	C	D

10) **Tartinabilité** :

1. Très difficile
2. Difficile
3. Moyenne
4. Facile
5. Très facile

A	B	C	D

11) Préférence globale :

Attribuez pour chaque échantillon une note de préférence entre 1 à 9, sachant que 1 correspond à l'échantillon le moins préféré et le numéro 9 à celui le plus préféré :

1. Extrêmement disagreeable
2. Très désagréable
3. Désagréable
4. Assez désagréable
5. Ni agréable ni désagréable
6. Assez agréable
7. Agréable
8. Très agréable
9. Extrêmement agréable.

A	B	C	D

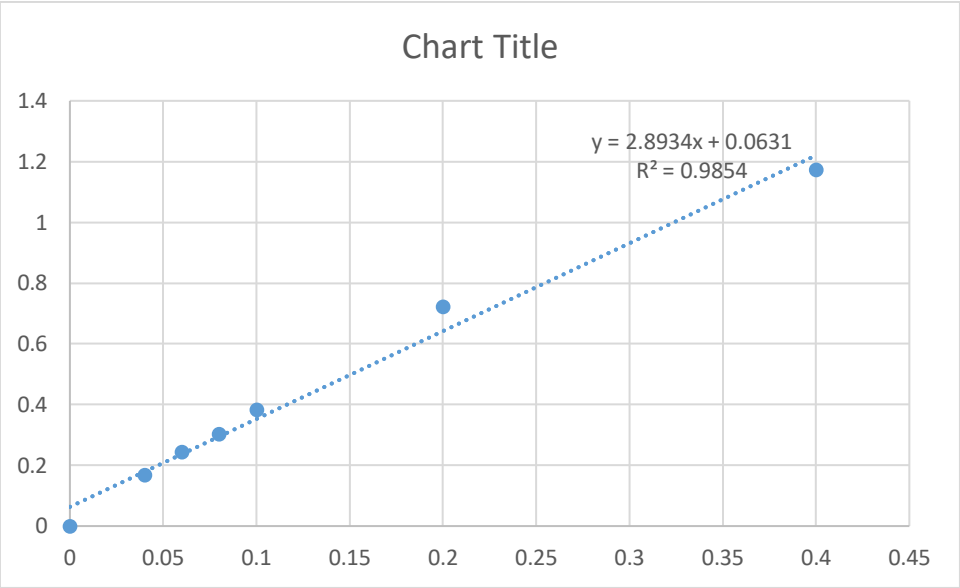


Figure 2: Gallic acid calibration curve.

The list of equipment used:

- centrifuge
- incubator
- stirring plate
- water bath
- balances
- ultrasonic tank.

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