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**TOPIC**

***Physicochemical characterization and  
nutritional value of quinoa Milk powder***

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# *Dedication*



*I am pleased to dedicate this work to:*

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# Introduction

### Introduction

Algeria ranks among the nations that have successfully embraced quinoa cultivation, owing to the scientific and technical guidance provided by the FAO. This assistance facilitated the assessment of quinoa's adaptability when it was first introduced to the country in 2013–2014. During this initial trial phase, eight experimental sites were selected to represent Algeria's diverse agro-ecological regions, including Bâinem (Algiers), Setif, Tiaret, Relizane, Guelma, Biskra, El Oued, and Adrar. Through international cooperation led by the FAO, a total of 16 quinoa genotypes were evaluated (Maamri;2022) .

Although milk provides significant dietary benefits, some individuals experience milk-related disorders, such as cow's milk allergy, which affects approximately 0.5% to 3.5% of the population, and lactose intolerance, which impacts 65% to 75% of individuals. Additionally, certain groups, such as vegetarians and vegans, choose to follow a milk-free diet. Given the widespread use of milk in daily meals and food products, as well as the growing number of people avoiding it, there is a clear need to develop suitable alternatives for daily consumption (fructuoso & al ;2021).

To address milk-related disorders, the primary alternatives to cow's milk include water-soluble extracts from various plant sources. These include legumes such as soybean (*Glycine max*) and chickpea (*Cicer arietinum*), nuts like almond (*Prunus dulcis*), cashew (*Anacardium occidentale*), hazelnut (*Corylus avellana*), and Brazil nut (*Bertholletia excelsa*), as well as seeds like sunflower (*Helianthus annuus*) and sesame (*Sesamum indicum*) (vilcacundo & Hernandez ledesma;2017).

Additionally, cereals such as rice (*Oryza spp.*) and oat (*Avena sativa*), along with pseudocereals like quinoa (*Chenopodium quinoa*), serve as milk alternatives. Consumers seek substitutes that closely resemble cow's milk in terms of color, texture, and, when possible, flavor. However, it is important to note that plant-based milk alternatives often do not match the nutritional profile of cow's milk ( fructuoso & al ; 2021 ) .

Given the rising importance of plant-based milks, our study focuses

specifically on quinoa milk powder. Quinoa has garnered significant global interest, highlighted by its designation as the International Year of Quinoa in 2013. The scientific community continues to explore its rich nutritional profile, which includes high-quality proteins, vitamins, lipids, fibers, and minerals. Quinoa is particularly valued for its well-balanced essential amino acids and potential health benefits, making it an excellent option for individuals with celiac disease and those seeking nutritious dietary alternatives.

This study has several complementary objectives. The first objective is to determine the optimal physicochemical parameters for the formulation of a stable quinoa beverage. The second objective is to evaluate the complete nutritional composition of the developed beverage. Finally, the third objective aims to characterize the organoleptic properties and biological activities of the final product.

This manuscript is structured in three chapters. The first chapter deals with synthesis bibliographical which revolves around vegetable drinks in particular of quinoa and also the importance of vegetable milk for people with a Milk intolerance of animal origin and also decreases the blood sugar index. The second chapter, addresses the preparation methods and physicochemical analyzes, the study of the nutritional value of quinoa milk . The third chapter presents the main results obtained discussions and interpretations. A general conclusion and prospects which are a Set of reflections that complement this work.

# **CHAPITRE I:**

## **Literature review**

### I.1. Definition

Plant-based beverages are fluids obtained through water extraction of plant materials, involving size reduction and homogenization. The resulting particles typically range from 5 to 20  $\mu\text{m}$ , creating a suspension that mimics the appearance of bovine milk ( [Fuentes & al; 2024](#) ).

Plant-based drinks lack a clear classification, both in terms of their definition and the nature of their colloidal system. These beverages can exhibit characteristics of emulsions, suspensions, or a combination of both. Their composition often includes dispersed plant cell fragments, soluble and insoluble proteins, lipids, and carbohydrates, leading to a complex system with diverse interactions([Patra & al ; 2022](#)).

### I.2. Types of plant based beverage :

Plant-based beverages can be classified into five main categories based on their source :

- **Legume beverages:** soy, peanut, pea, lupin, and cowpea;
- **Nut beverages:** almond, coconut, hazelnut, pistachio, walnut, and cashew;
- **Cereal beverages:** oat, rice, corn, and spelt;
- **Pseudo-cereal beverages:** quinoa, teff, and amaranth;
- **Seed beverages:** sesame, flaxseed, hemp, sunflower.

### I.3. Nutrients composition of plants based milks :

Plant-based milks (PBMs) vary significantly in their nutrient composition compared to dairy milk. In terms of macronutrients, PBMs generally contain less energy, except for coconut milk, which has a high fat content. Soy and pea milks provide protein levels comparable to dairy milk, whereas oat, almond, and rice milks have significantly lower protein content. Carbohydrate levels also vary, with oat and rice milks containing the highest amounts, even exceeding dairy milk. Unlike dairy milk, PBMs contain dietary fiber. Regarding fats, most PBMs are lower in fat compared to dairy milk, except for coconut milk, which is rich in saturated fats. ([J berdy&al ;2022](#))

PBMs primarily contain unsaturated fats, while dairy and coconut milk have higher levels of saturated fats, and only dairy milk contains cholesterol. In terms of minerals, PBMs often contain less calcium than dairy milk, though fortified versions can compensate for this. Soy and coconut milks are higher in magnesium, while potassium levels in PBMs are generally lower, except for soy milk, which is comparable to dairy. Sodium levels vary depending on added salt during production. PBMs also tend to have lower amounts of vitamin B-12 and vitamin D compared to dairy milk, unless fortified, but

soy and almond milks are richer in folate and vitamin E. Some PBMs, such as soy milk, contain bioactive compounds like isoflavones and phytosterols, which may offer additional health benefits. The bioavailability of added nutrients in PBMs may differ from those naturally found in dairy milk, highlighting the importance of fortification to ensure nutritional adequacy. (Berdy & al ;2022)

**Tableau 1 : main bioactive compounds of selected plant-based milk alternative and their health benefits (JURADO & al;2021)**

PBMAs	Bioactive compound	Health benefits
<b>Almond milk</b>	<ul style="list-style-type: none"> <li>• <math>\alpha</math>-tocopherol</li> <li>• Arabinose</li> <li>• Flavonoïde and phytosterol</li> </ul>	<ul style="list-style-type: none"> <li>➤ Lowers plasma LDL cholesterol level</li> <li>➤ Decreases lipid per-oxidation</li> <li>➤ Improves gastrointestinal health</li> <li>➤ Prebiotic properties</li> </ul>
<b>Coconut milk</b>	<ul style="list-style-type: none"> <li>➤ Lauric acid</li> <li>➤ Medium chain triglycerides</li> </ul>	<ul style="list-style-type: none"> <li>➤ Promotes brain development</li> <li>➤ Maintains the elasticity of blood vessels</li> <li>➤ Boosts immune system</li> </ul>
<b>Soybean milk</b>	<ul style="list-style-type: none"> <li>➤ Isoflavones</li> <li>➤ phytosterol</li> <li>➤ <math>\alpha</math>-tocopherol</li> </ul>	<ul style="list-style-type: none"> <li>➤ Decreases the LDL cholesterol and increases HDL cholesterol [3,18] Soybean milk alternative Isoflavones Phytosterol <math>\alpha</math>-tocopherol</li> <li>➤ Ability to bind with estrogen receptors and develop properties like the estrogenic</li> <li>➤ Alleviate menopause symptoms</li> <li>➤ Decreases the risk of breast, prostate and colon cancer</li> <li>➤ Protective effect against osteoporosis and cardiovascular diseases</li> <li>➤ Properties of lowering cholesterol</li> <li>➤ Functions as an anti-inflammatory agent</li> </ul>
<b>Rice milk</b>	<ul style="list-style-type: none"> <li>➤ Phytosterols, especially <math>\beta</math>sitosterol and <math>\gamma</math>-oryzanol</li> </ul>	<ul style="list-style-type: none"> <li>➤ Reduction of cholesterol levels</li> <li>➤ Lowers hypertension</li> <li>➤ Anti-diabetic properties</li> <li>➤ Anti-inflammatory properties</li> <li>➤ Antioxidant activity</li> </ul>
<b>Cashew milk</b>	<ul style="list-style-type: none"> <li>➤ Phytosterols, polyunsaturated fats</li> </ul>	<ul style="list-style-type: none"> <li>➤ Lowers LDL cholesterol by inhibiting cholesterol biosynthesis</li> </ul>
<b>Oat milk</b>	<ul style="list-style-type: none"> <li>➤ <math>\beta</math>-glucan and phytosterols</li> </ul>	<ul style="list-style-type: none"> <li>➤ Delay the time of gastric emptying</li> <li>➤ Reduction of postprandial glycemic response because of the increasing of gastrointestinal transit</li> <li>➤ Reduction of total and LDL cholesterol</li> <li>➤ Management of body weight and blood pressure</li> </ul>
<b>Sesame milk</b>	<ul style="list-style-type: none"> <li>➤ Lignans (sesamin, sesamol, and sesaminol)</li> </ul>	<ul style="list-style-type: none"> <li>➤ It has hypocholesterolemic activity</li> <li>➤ Antitumor activity</li> <li>➤ Antiviral activity</li> </ul>
<b>Peanuts milk</b>	<ul style="list-style-type: none"> <li>➤ Phenolic compounds</li> </ul>	<ul style="list-style-type: none"> <li>➤ Protective role against oxidative damage and diseases like coronary heart disease, stroke, and various cancers</li> </ul>

#### I.4. Comparison between animal milk and plant-based drinks effects on the intestinal micro-biota :

##### 4.1. Effect of Animal-Based Milk on the Gut Microbiota :

animal-based milk positively influences the gut microbiota due to its bioactive components, including proteins, lipids, and oligosaccharides. Studies show that consuming animal milk increases gut microbiota richness and diversity, enhances the production of short-chain fatty acids (SCFAs), and promotes beneficial bacteria like *Bifidobacterium*, *Lactobacilli*, *Akkermansia*, *Lachnospiraceae*, and *Blautia*. It also reduces harmful bacteria such as *Proteobacteria*, *Erysipelotrichaceae*, *Desulfovibrionaceae*, and *Clostridium perfringens*. Key components of milk, including whey proteins, oligosaccharides, and the milk fat globule membrane (MFGM), contribute to these effects by supporting probiotic growth and inhibiting pathogens. Additionally, variations in milk composition across species (cow, goat, camel, etc.) impact microbiota differently, with camel milk showing strong prebiotic and immunomodulatory properties. .( [Mondragon&al;2024](#)).

**Tableau 2 :Effects of animal milk components on human gut microbiota (Mondragon & al,2024).**

Effects on Gut Microbiota	
<b>Lactose</b>	Increase lactobacilli and <i>Bifidobacterium</i> growth
<b>Milk oligosaccharides</b>	Favors growth of beneficial bacteria as lactobacilli and <i>Bifidobacterium</i> ; Inhibiting bacterial adhesion of pathogens to enterocytes
<b><math>\alpha</math>-lactalbumin</b>	Promoting growth of beneficial bacteria and exerts antimicrobial activity against some pathogens
<b>Lactoferrin</b>	Prebiotic effect and inhibition of pathogens
<b>Lysozyme</b>	increase resistance to intestinal colonization by some pathogens
<b>Milk fat globule membrane</b>	Promote the formation of binding groups on the surface of probiotics; In vitro bactericidal effects against some pathogens

#### 4.2. Effect of vegetable -Based Milk on the Gut Microbiota :

Vegetable-based milk influences gut microbiota in both beneficial and potentially harmful ways. It promotes the growth of beneficial bacteria like *Lactobacilli*, *Bifidobacterium*, and *Blautia*, while also providing an alternative for lactose-intolerant individuals and vegans due to its lack of lactose and cholesterol. Additionally, its bioactive compounds, such as polyphenols and  $\beta$ -glucans, may support gut health. However, some studies indicate a reduction in key probiotics like *Lactobacilli* and *Bifidobacterium*, along with an increase in potentially harmful bacteria such as *Enterobacteriaceae*, *Salmonella*, and *Fusobacterium*, which is linked to colon cancer. The effects on the Firmicutes/Bacteroidetes ratio vary, sometimes suggesting a connection to obesity. While plant-based milk can be a healthy alternative, it does not fully replicate the positive impact of animal milk on gut microbiota, requiring further research to assess its long-term effects. (Mondragon & al,2024).

#### I.5. Nutritional disadvantage of vegetal beverage :

Although plant-based dairy alternatives are increasingly popular due to health, ethical, and environmental considerations, they present notable nutritional disadvantages compared to cow's milk. One major concern is their generally lower protein content and inferior amino acid profiles, which may not fully support human dietary needs particularly in options like rice milk. Additionally, while many of these beverages are fortified with essential nutrients such as calcium and vitamin D, the bioavailability of these nutrients is often reduced due to natural compounds like phytates that inhibit absorption. (Ismail;2015) Another issue is the lack of standardization in fortification practices, leading to considerable variability in nutritional content across different brands. Furthermore, many commercial plant-based milks contain added sugars, flavorings, stabilizers, and preservatives, which can detract from their nutritional quality and increase calorie intake. (Mondragon & al,2024). These limitations are especially important to consider for vulnerable populations such as young children or individuals who rely heavily on these products who may face increased risk of deficiencies in protein, calcium, vitamin B12, and other vital nutrients if these beverages are not carefully integrated into a balanced diet. (JANCUROVÁ & al,2009)

## I.7. Quinoa :

### 7.1. Botanical description of quinoa :

Wilson;1990 provided a detailed botanical classification of cultivated *Chenopodium* species. Initially placed in the *Chenopodiaceae* family, the genus *Chenopodium* was later reclassified under *Amaranthaceae* following a phylogenetic revision (Angiosperm Phylogeny Group, 1998). Domesticated *Chenopodium* species are categorized into two subsections: *Cellulata* and *Leiosperma*. The *Leiosperma* group includes the South American *cañihua* (*C. pallidculale*) and the Eurasian *C. album*, while *Cellulata* comprises *quinoa* (*C. quinoa*) and *huazontle* (*C. berlandieri* subsp. *nuttalliae*).  
(Maughan&al;2007)

Quinoa exhibits epigeal germination, meaning its cotyledons emerge above the soil surface. The leaves are thick, fleshy, and smooth, arranged alternately with petiolate structures. They vary in shape, with broad, rhomboid, or triangular forms at the lower stem, becoming small and lanceolate near the inflorescence. The leaf surface contains calcium oxalate crystals, giving it a gritty texture.

The stem develops significantly after two weeks, growing 70–100 cm tall, with a thick, green, cylindrical base that becomes angular due to leaf arrangements. It has a cutinized epidermis, providing structural strength.

The inflorescence appears around the seventh week, forming a compound raceme (panicle) with multiple branching axes. It has an amaranth-like structure, as described by Cronquist ;1981.

Flowers are small, green, and hypogynous , with a simple perianth. They are hermaphroditic and self-fertilizing, lacking petals. The five green sepals contain calcium oxalate crystals and have vascular bundles. The androecium consists of five stamens with short filaments and four-loculed anthers. The gynoecium has a superior ovary with a feathery, branched stigma and a single basal ovule.

The fruit is a small seed enclosed in a thin pericarp, containing an embryo with two cotyledons, a plumule, and a radicle. The seed surrounds the perisperm, which serves as the main starch storage tissue. Additionally, the seed has a cellular endosperm that provides nutrients to the developing embryo.  
(Goma;2014)



**Figure 1 : The morphological shapes of different part of *Chenopodium quinoa* plant.( Goma;2014)**

## 7.2. Varieties of quinoa:

The earliest archaeological evidence of domesticated quinoa dates back to 5000 BC. *Chenopodium quinoa Willd.* has long been a staple food in Andean South America, where it was domesticated by ancient civilizations in the Bolivian and Peruvian Altiplano. Genetic diversity studies have shown that the highest variation in quinoa landraces is concentrated in the region between Cuzco, Peru, and Potosí, Bolivia, with the greatest number of landraces found around Lake Titicaca. (Jacobsen *et al*;2005) There is a general consensus that quinoa originated in the Andean Altiplano, with its historical cultivation spreading across Bolivia, Peru, Ecuador, northern Chile, and Colombia. Currently, 26 genebanks in South America preserve quinoa genetic resources, with 24 of them located in Andean countries ( belgust&al;2024).

**Tableau 3 : Varieties of quinoa (hakim&al.2021)**

Category	Variety Name	Characteristics	Origin
<b>By Color</b>	<b>White Quinoa</b>	Mild flavor, fluffy texture, balanced protein and carbohydrates	Common worldwide
	<b>Red Quinoa</b>	Firmer texture, nutty taste, high in antioxidants	South America
	<b>Black Quinoa</b>	Earthy flavor, firm texture, highest antioxidant content	South America
	<b>Tricolor Quinoa</b>	Blend of white, red, and black quinoa for a balanced texture and color	Mixed
<b>By Geographic Origin</b>	<b>Andean Quinoa</b>	Grown in high altitudes, adapted to cold climates	Bolivia, Peru, Ecuador, Chile
	<b>Altiplano Quinoa</b>	Larger seeds, resistant to extreme conditions	Bolivia, Peru
	<b>Inter-Andean Valley Quinoa</b>	Grows at lower altitudes, shorter maturation period	Peru, Ecuador
	<b>Coastal Quinoa</b>	Cultivated in temperate coastal regions	Chile
<b>Improved &amp; Commercial Varieties</b>	<b>Quinoa Real</b>	Large seeds, high nutritional value, premium export quality	Bolivia
	<b>Titicaca</b>	Cold-resistant, suitable for European climates	Denmark
	<b>Puno</b>	Shorter growing season, adapted to temperate climates	Developed for global adaptation
	<b>Cherry Vanilla &amp; Brightest Brilliant Rainbow</b>	Colorful varieties, used for ornamental and culinary purposes	Various regions

## 7.2. Nutritive composition of quinoa seeds :

### 7.2.1. Protein :

Quinoa seeds are recognized for their high protein content, making them a valuable source of plant-based nutrition. The protein content in quinoa typically ranges between 12% and 16%, depending on the variety and growing conditions. Unlike many other cereals, quinoa contains a balanced profile of essential amino acids, closely resembling the amino acid composition of casein, a protein found in milk. (jancurova.M&al.2009)

Amino acids such as lysine, methionine, and tryptophan are present in significant amounts, which is particularly beneficial since lysine is often deficient in other grains. The protein digestibility of quinoa is also high, making it an excellent alternative for those seeking high-quality plant proteins. Due to these properties, quinoa has gained attention as an ideal protein

**Tableau 4 : amino acide content. (jancurova.M&al.2009)**

<b>Amino Acid</b>	<b>Content (%)</b>
<b>Lysine</b>	5.1 - 6.4
<b>Methionine</b>	2.1 - 2.8
<b>Tryptophan</b>	1.0 - 1.3
<b>Leucine</b>	6.8 - 8.0
<b>Isoleucine</b>	4.0 - 4.9
<b>Valine</b>	4.5 - 5.3
<b>Phenylalanine</b>	4.6 - 5.2
<b>Threonine</b>	3.7 - 4.4
<b>Histidine</b>	2.5 - 3.2

### 7.2.2. Carbohydrates :

constitute the major component of quinoa seeds, accounting for approximately 58% to 64% of the total dry weight. The primary carbohydrate present in quinoa is starch, which represents around 52% to 60% of the seed's dry matter. Quinoa starch is characterized by a small granule size (1-3  $\mu\text{m}$ ), making it more digestible compared to starches from other cereals. . (jancurova.M&al.2009)

In addition to starch, quinoa contains dietary fiber, including both soluble and insoluble fractions, which contribute to its health benefits. The total dietary fiber content in quinoa ranges between 7% and 10%, with insoluble fiber making up the majority. This high fiber content supports digestive health, glycemic control, and satiety, making quinoa a favorable ingredient for health-conscious consumers. . (jancurova.M&al.2009)

Another significant carbohydrate fraction in quinoa is soluble polysaccharides, which may contribute to its unique texture and viscosity in food applications such as plant-based milk and yogurt. Additionally, quinoa has a low glycemic index (GI) compared to traditional cereals, making it a suitable choice for individuals with diabetes or those following low-GI diets. source for vegetarian and vegan diets, as well as for individuals with gluten intolerance.

**Tableau 5 : carbohydrate content. (jancurova.M&al.2009)**

Carbohydrate Component	Content (%)
Total Carbohydrates	58 - 64
Starch	52 - 60
Total Dietary Fiber	7 - 10
Insoluble Fiber	5 - 7
Soluble Fiber	1 - 3
Sugars	1 - 2

### 7.2.3. Vitamins and Minerals

Quinoa seeds are an excellent source of essential vitamins and minerals, contributing to their high nutritional value. Compared to traditional grains, quinoa contains higher levels of iron, magnesium, phosphorus, and zinc, making it a valuable dietary option for individuals with nutritional deficiencies. . (jancurova.M&al.2009)

### 7.2.3.1. Mineral Composition

Quinoa is particularly rich in magnesium (Mg), an essential mineral involved in muscle function, nerve signaling, and bone health. The iron (Fe) content is also relatively high, supporting red blood cell production and oxygen transport. Additionally, quinoa provides significant amounts of calcium (Ca), potassium (K), zinc (Zn), and phosphorus (P), all of which play vital roles in maintaining overall health. . (jancurova &al.2009)

**Tableau 6 : mineral content. (jancurova &al.2009)**

Mineral	Content (mg/100g)
Magnesium (Mg)	150 - 200
Iron (Fe)	4 - 6
Calcium (Ca)	40 - 80
Potassium (K)	500 - 700
Zinc (Zn)	2 - 3.5
Phosphorus (P)	280 - 400
Manganese (Mn)	1 - 2.5
Copper (Cu)	0.5 - 1.2

### 7.2.3.2. Vitamin Composition

Quinoa also contains important vitamins, particularly those from the B-complex group, which are crucial for energy metabolism and nervous system function. Among these, vitamin B1 (thiamine), B2 (riboflavin), B3 (niacin), and B6 (pyridoxine) are found in substantial amounts. Additionally, quinoa contains vitamin E, a powerful antioxidant that supports skin health and immune function. . (jancurova &al.2009)

**Tableau 7 : vitamin content. (jancurova.M&al.2009)**

Vitamin	Content (mg/100g)
Vitamin B1 (Thiamine)	0.3 - 0.5
Vitamin B2 (Riboflavin)	0.2 - 0.4
Vitamin B3 (Niacin)	0.5 - 1.0
Vitamin B6 (Pyridoxine)	0.3 - 0.6
Folate (B9)	40 - 100 µg

Vitamin E	0.6 - 2.0
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#### 7.2.4. Lipid

Quinoa seeds contain a moderate amount of fat, typically ranging from 4% to 9% of the seed's total weight. Despite its relatively low lipid content compared to oilseeds, quinoa stands out for its high proportion of unsaturated fatty acids, which contribute to its health benefits. The lipid profile of quinoa consists mainly of polyunsaturated fatty acids (PUFAs), monounsaturated fatty acids (MUFAs), and saturated fatty acids (SFAs), making it a valuable source of essential fatty acids. . (jancurova &al.2009)

##### 7.2.4.1. Fatty Acid Composition

The predominant fatty acids found in quinoa include linoleic acid ( $\omega$ -6) and  $\alpha$ -linolenic acid ( $\omega$ -3), both of which are essential for heart health, brain function, and anti-inflammatory responses. Quinoa also contains notable amounts of oleic acid ( $\omega$ -9), which is known for its role in promoting cardiovascular health. The omega-6 to omega-3 ratio in quinoa is relatively balanced, making it a beneficial dietary option for maintaining an optimal fatty acid profile. . (jancurova &al.2009)

**Tableau 8 : fatty acid content. (jancurova&al.2009)**

Fatty Acid	Content (%)
Total Lipids	4 - 9
Saturated Fatty Acids (SFAs)	10 - 15
Monounsaturated Fatty Acids (MUFAs)	20 - 30
Polyunsaturated Fatty Acids (PUFAs)	50 - 60
Linoleic Acid ( $\omega$ -6)	40 - 50
$\alpha$ -Linolenic Acid ( $\omega$ -3)	4 - 8
Oleic Acid ( $\omega$ -9)	20 - 25

##### 7.2.4.2. Lipid Stability and Antioxidant Content

One of the unique features of quinoa lipids is their stability, which is enhanced by the presence of natural antioxidants, such as vitamin E (tocopherols) and polyphenols. These compounds help protect the fatty acids from oxidation, prolonging the shelf life of quinoa-based products and preserving their nutritional quality.

Due to its rich unsaturated fatty acid profile, quinoa is considered a healthy alternative to traditional grains. Its lipid composition makes it an ideal ingredient for

functional foods, plant-based dairy alternatives, and formulations aimed at improving cardiovascular health. (Jancurova & al. 2009)

### 7.3. Traditional use of quinoa:

Quinoa (*Chenopodium quinoa*) is a plant native to the Andes, where it has been cultivated for millennia. Its traditional uses are varied and deeply rooted in Andean cultures. Here are some of these uses:

- **Boiled seeds:** Quinoa seeds were traditionally consumed in the form of soups or porridges, providing an essential source of nutrients for Andean populations.
- **Flour and breads:** Quinoa was ground into flour for the preparation of flatbreads and breads, providing a nutritious alternative to other grains.
- **Traditional beverages:** Fermented beverages, such as chicha de quinoa, were made from the seeds, playing an important role in ceremonies and rituals.
- **Digestive remedy:** Quinoa infusions were consumed to treat various digestive disorders, attesting to its importance in traditional medicine.
- **Animal fodder:** The parts not consumed by humans, such as stems and leaves, were used as food for livestock, especially llamas and alpacas, demonstrating the integral use of the plant.
- **Ritual and cultural practices:** Quinoa occupied a central place in the rituals and ceremonies of Andean civilizations, symbolizing fertility and prosperity. The first harvests were often dedicated to the deities, reflecting its spiritual importance.

### 7.3. Economic Importance of Quinoa :

- **In the world :**

Quinoa (*Chenopodium quinoa* Willd.) originally from the Andean region, is now gaining significant global economic value due to its exceptional nutritional properties—particularly its high content of complete proteins, fiber, essential amino acids, minerals, and antioxidants. This nutritional potential led the FAO to declare 2013 the "International Year of Quinoa," recognizing the crop as a potential pillar of global food security (FAO, 2013). Additionally, collaborations such as the joint FAO/IAEA program aim to enhance quinoa's yield and resilience under diverse agricultural conditions (FAO/IAEA, 2020). Economically, quinoa is increasingly in demand in Western food industries, where it is integrated into high-value processed products (FAO, 2013).

- **In Algeria :**

Quinoa is emerging as a strategic crop, especially suited to arid and Saharan regions due to its tolerance to salinity, drought, and high temperatures. Several agronomic trials have been conducted in regions such as Ouargla, Adrar, and El Oued, showing promising adaptation to local agroclimatic conditions and strong yield potential (**Hamouda & al., 2022**). At the same time, valorization initiatives are underway, particularly in Oued Souf, where experimental trials by the Technical Institute for the Development of Saharan Agriculture have demonstrated the crop's viability in poor, sandy, and saline soils (**Benkahla & al., 2023**). Moreover, studies by the INRAA (National Institute of Agronomic Research of Algeria) in Relizane and Adrar have shown quinoa's potential to contribute to the diversification of national agricultural systems (**Gacemi & al., 2020**).

## **&I.6.Quinoa milk :**

### **6.1. Definition :**

Quinoa milk is a plant-based alternative to dairy, created by blending quinoa with water and then straining out the solids. The result is a smooth, creamy beverage that serves as a nutritious substitute for traditional milk.

Quinoa milk is a plant-based beverage made from quinoa seeds (*Chenopodium quinoa Willd.*), usually through a process of soaking, grinding, filtering, and sometimes enriching with nutrients. It is a lactose-free alternative to animal milks and is valued for its richness in protein, fiber, essential amino acids, and minerals such as iron, magnesium, and calcium. (**Mäkinen&al.2016**)

### **6.2. The effects of quinoa powder as a substitute for skim milk powder in yogurt:**

Quinoa powder has shown promising potential as a replacement for skim milk powder in yogurt production. Its inclusion enhances the nutritional profile of yogurt by increasing protein, fat, and total solids, while also contributing essential amino acids and dietary fiber. Functionally, it improves water-holding capacity and reduces syneresis, resulting in more stable and thicker yogurts (**Fructuoso;2021**). Rheological properties such as viscosity, firmness, and consistency are also improved, particularly at higher substitution levels. Sensory evaluations indicate that quinoa flour can replace skim milk powder by up to 75% without negatively affecting taste, texture, or appearance, making it a valuable ingredient for producing nutritionally enriched and high-quality yogurt products. (**Alkobeisi&al.2022**) .

### 6.3. Therapeutic benefits of quinoa milk :

#### 6.3.1. Glycemia index :

The glycemic index (GI) is defined as the incremental area under the glucose response curve following the consumption of a standardized amount of carbohydrates from a test food, compared to a reference food such as white bread or glucose. The GI of a particular food or meal is primarily influenced by the type of carbohydrates it contains, along with other dietary factors that impact nutrient digestibility and insulin secretion (Ludwing;2002).

Quinoa milk, derived from quinoa grains, offers a unique nutritional profile that influences its glycemic response. With quinoa being classified as a medium glycemic index (GI) food, its conversion into milk retains its balanced carbohydrate composition while benefiting from its high-quality protein and essential fatty acids. The presence of complete proteins in quinoa milk slows digestion and gastric emptying, contributing to a more gradual release of glucose into the bloodstream compared to other plant-based milks like rice milk, which typically have a higher GI. Additionally, the omega-3 and omega-6 fatty acids in quinoa milk, along with its dietary fiber content, further modulate the glycemic response by reducing glucose absorption. As a result, quinoa milk can be considered a preferable alternative for individuals seeking blood sugar control, particularly those managing diabetes or looking for sustained energy release. However, while quinoa milk has a lower glycemic impact than certain alternatives, portion size remains a key factor in maintaining stable blood sugar levels (M.pryanka&al;2018).

#### 6.3.2. Lactose and gluten intolerance :

A recent NIH conference redefined lactose intolerance as the occurrence of gastrointestinal symptoms following a blinded, single-dose lactose challenge in an individual with lactose malabsorption, while no symptoms appear when the same person consumes an indistinguishable placebo. This revised definition is notable because it not only requires confirmation of lactase deficiency or lactose malabsorption but also mandates symptom development under controlled, placebo-blinded conditions—an approach that is not yet standard clinical practice ( Misselwitz&al.2013).

Gluten intolerance is an immune-mediated enteropathy triggered by gluten-containing foods in individuals with genetic susceptibility. In its typical form, primarily seen in children, it manifests as failure to thrive and/or gastrointestinal symptoms. In adults, the condition presents less distinctly, often causing mild digestive discomfort, iron

deficiency (with or without anemia), or nonspecific abnormalities in serum chemistry ( **MJ Llorente-Alonso MD&al.2006**).

Quinoa offers significant benefits to high-risk consumer groups, including children, the elderly, high-performance athletes, individuals with lactose intolerance, women at risk of osteoporosis, and those with anemia, diabetes, dyslipidemia, obesity, or celiac disease. Its high nutritional value, therapeutic properties, and gluten-free nature make it a superior choice for health and well-being. These benefits are primarily attributed to quinoa's rich composition of fiber, minerals, vitamins, fatty acids, antioxidants, and, most notably, phytochemicals, which set it apart from other crops in human nutrition ( **Subeeth&al.2019**).

### 6.3.3. Celiac disease :

**Zevallos&al. (2014)** conducted a study involving nineteen celiac patients who consumed 50 g of quinoa per day for six weeks as part of their regular gluten-free diet. During the study, duodenal biopsies and blood samples were collected to assess the effects of quinoa consumption.

The results indicated that quinoa was well-tolerated and did not worsen the clinical symptoms of celiac disease. Additionally, the study revealed a positive trend in histological improvements:

- The villus height to crypt depth ratio increased from 2.8:1 (slightly below normal) to 3:1 (normal level).
- The surface-enterocyte cell height improved from 28.76  $\mu\text{m}$  to 29.77  $\mu\text{m}$ .
- The number of intraepithelial lymphocytes per 100 enterocytes slightly decreased from 30.3 to 29.7 (**Pakbaz&al,2021**).

### 6.3.4. Betaine and metabolic syndrome :

Cross-sectional data in humans indicate a significant negative correlation between obesity markers (BMI, body fat percentage, and waist circumference) and plasma betaine levels. Additionally, over 20% of diabetes mellitus patients exhibit abnormally high betaine excretion in urine. Higher plasma betaine concentrations have also been linked to lower triglycerides (TG), homocysteine levels, and inflammation markers (**Pakbaz&al 2021**).

Quinoa stands out as a particularly beneficial food for celiac patients, serving as a key component of a healthy, gluten-free diet. Moreover, it is hypothesized that incorporating quinoa into the diet could help reduce oxidative stress, improve lipid profiles, aid in weight and glucose control, and lower risk factors for cardiovascular disease and type 2 diabetes. Quinoa may even have therapeutic potential in reversing some effects of these conditions. However, despite these promising attributes, there is still a lack of extensive scientific research, as only a few studies—whether in vitro, in vivo, or clinical trials—have been conducted to establish strong translational applications based on robust scientific evidence ( [Pakbaz & al ,2021](#)).

# **Chapter II :**

## **Material and methods**

**1. Preparation of quinoa-based vegetable drink :**

To make a traditional quinoa-based plant-based milk, start by thoroughly rinsing one cup of quinoa grains under cold water to remove the saponins, which are responsible for the bitterness. . Blend the quinoa with four cups of water for 2 to 3 minutes until smooth. Then strain the mixture through a clean tea towel or cheesecloth to remove the pulp and obtain a smooth milk. it into a clean glass bottle, store it in the refrigerator and consume it within 3 to 4 days. ([simpleveganblog.com](http://simpleveganblog.com))

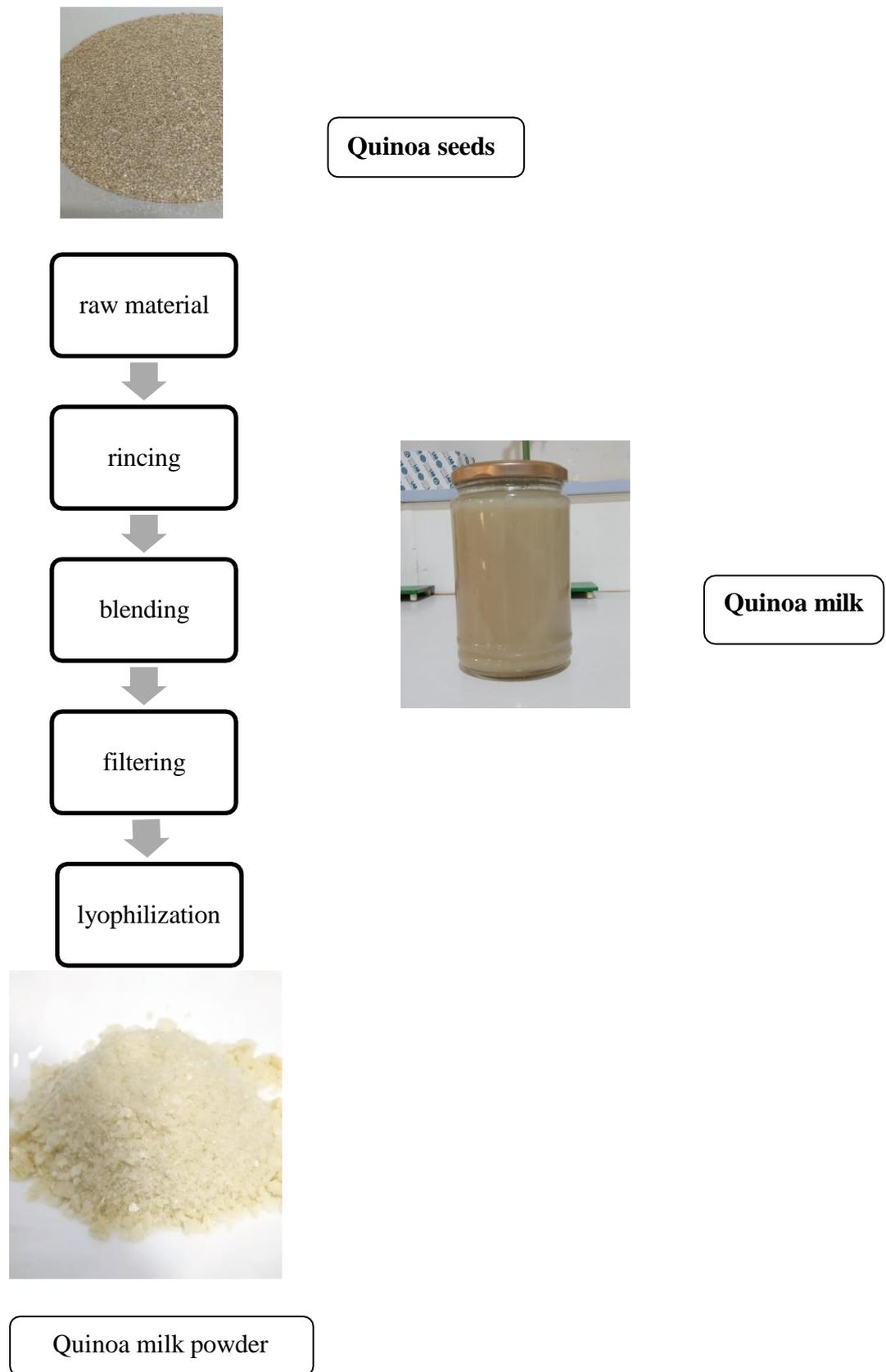


Figure 2 : steps of preparation of sample

## 2. Organoleptic analysis :

The sensory evaluation of the samples was performed following a hedonic protocol set by **Stone and Sidel (2004)**. The samples were evaluated by 30 untrained panelists, 18 to 60 years old. They evaluated different samples in a quiet, ventilated room without extraneous odors at random order. The quinoa milk were evaluated by each panelist on four sensory attributes: color, odor, texture, taste. A five-point hedonic scale from 1 (do not like very much) to 5 (like very much) was used. The values were recorded on an individual assessment sheet. Mouth rinsing with water between the samples was asked for to avoid sensory satiation. The values obtained were statistically processed and calculated based on the means, and then plotted on a radar chart. This protocol also conforms to the ISO 13299:2016 standard for descriptive sensory analysis recommendations.

## 3. The physicochemical analyses carried out :

### 3.1. pH Measurement :

pH is a quantitative measure of the acidity or basicity of a solution. It is a parameter used to determine the concentration of hydrogen ions ( $H^+$ ) in a solution. (**Cachau-Herreillat, 2009**).

#### 3.1.1. Operating Procedure :

Calibrate the pH meter using distilled water and verify that the device is functioning properly. Pour a small but sufficient volume of the sample into a beaker to allow complete immersion of the electrode.

**Measurement:** The pH value of the sample is read directly from the pH meter display. Perform two measurements on the same sample. Between each measurement, rinse the electrode with distilled water and dry it using absorbent paper.

#### 3.1.2. Expression of Results :

The pH value is read directly from the device. Record the arithmetic mean of the two measurements. If the difference between the two readings is too large, a third measurement should be taken.

### 3.2. Determination of Ash Content :

To determine the ash content, a known volume (10 mL) of quinoa milk is placed in pre-weighed porcelain crucibles. These crucibles are first dried in an oven and then incinerated in a muffle furnace at 500°C for 4 hours, until white or grey ash is obtained. After incineration, the crucibles are cooled in a desiccator and then weighed precisely along with their ash residues. (**BARKHATOV & ELISSEV, 1979**).

**Expression of Results:**

- $X_0$  : Mass of the empty crucible
- $X_1$  : Mass of the crucible with the quinoa milk sample
- $X_2$  : Mass of the crucible with ash residue

$$\text{Cendres}\% = (X_2 - X_0 / X_1) \times 100$$

**3.3. Moisture Content :****3.3.1. Principle :**

The sample is heated in a ventilated oven at  $100^\circ\text{C} \pm 5^\circ\text{C}$  until its weight becomes constant. The loss in weight corresponds to the evaporation of moisture from the sample.

**3.3.2. Procedure:**

1. Weigh the empty crucible (mass =  $m_0$  ). Ensure that the crucible has been properly dried in the oven, then allowed to cool in a desiccator.
2. Using a syringe, deposit 5 ml of the sample into the crucible. Weigh the filled crucible (mass =  $m_1$  ).
3. Place the crucible in a water bath at  $95^\circ\text{C}$  to pre-dry the sample.
4. Transfer the crucible to an oven set at  $100^\circ\text{C}$  and heat until the mass becomes constant (indicating complete dehydration).
5. Allow the crucible to cool in a desiccator to prevent moisture absorption from the air.
6. Weigh the crucible again after dehydration (mass =  $m_2$  ).

**3.3.3. Expression of Results :**

- $m_0$  : mass of the empty crucible
- $m_1$  : mass of the crucible with the sample before drying
- $m_2$  : mass of the crucible after drying

The moisture content (%) can be calculated using the formula:

$$\text{H}\% = (M_1 - M_2 / P) \times 100\%$$

**3.4. Total Phenolic Content Assay :**

The total phenolic content was determined using the Folin-Ciocalteu method described by (Singleton and Rossi1999). This method is based on the reduction of the phosphotungstic acid and phosphomolybdic acid mixture in a basic medium by the oxidizable groups of the phenolic compounds present in the sample. The reduction

products are tungsten and molybdenum metal oxides, which produce a blue color. A volume of 0.2 ml of the milk or standard (gallic acid) is mixed with 1.3 ml of the Folin-Ciocalteu reagent (diluted 1/10 with distilled water). After resting for 5 minutes, 1 ml of a  $\text{Na}_2\text{CO}_3$  (7.5%) solution is added. After thorough mixing, the sample is allowed to rest for 60 minutes in the dark at room temperature. The absorbance is then measured at 765 nm using a UV-Visible spectrophotometer,

### 3.5. Determination of protein content:

The Biuret method is a colorimetric technique commonly used to determine the concentration of proteins in a solution. It relies on the formation of a violet-colored complex when proteins react with copper sulfate in an alkaline environment, which can be quantified by measuring absorbance at 540 nm using a spectrophotometer. To perform this assay, a Biuret reagent is prepared by dissolving 1.5 g of copper sulfate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ), 6 g of sodium-potassium tartrate, and 300 mL of 0.3 M sodium hydroxide in distilled water, and diluting the mixture to a final volume of 1 liter. A standard protein solution, such as bovine serum albumin (BSA), is used to generate a calibration curve by preparing a range of concentrations (e.g., 0, 50, 100, 200, 400, 600, and 800 mg/L), with 1 mL of each standard added to separate test tubes. Likewise, 1 mL of each test sample is placed into individual tubes. To all tubes, 3 mL of Biuret reagent is added, followed by gentle mixing and incubation at room temperature for 30 minutes. After incubation, the absorbance of each solution is measured at 540 nm, with distilled water serving as a blank. The resulting absorbance values are then compared to the standard curve to determine the protein concentration in the samples.

### 3.6. Determination of flavonoids content :

Flavonoid determination was performed using the colorimetric method of (Kim *et al*;2003). A volume of 0.5 ml of quinoa milk was diluted with 0.5 ml of distilled water. Then, 0.3 ml of sodium nitrite solution (5%) was added. After 5 min, 0.3 ml of aluminum chloride solution (10%) was added. The mixture was allowed to stand for 5 min, then 2 ml of sodium hydroxide solution (1 M) was added. After stirring, absorbance was immediately measured at 510 nm using a UV-Visible spectrophotometer. quercetin was used as a standard with concentrations ranging from 0 to 500  $\mu\text{g}/\text{ml}$ , and the results are expressed as mg quercetin equivalent/g of dry weight of quinoa milk.

### 3.7. Determination of fat content :

The beverage's constituents other than fat are dissolved by sulfuric acid, and

thanks to centrifugal force and the addition of a small amount of isoamyl alcohol which dissolves the fat, the fat separates and rises to the top of the butyrometer. The analysis must be performed under a fume hood. Using a graduated pipette, 10 mL of sulfuric acid (91%) is carefully introduced into a butyrometer by allowing the tip of the pipette to touch the base of the butyrometer neck; this step aims to dissolve all components of the sample except the fat. Then, 5 mL of the plant-based beverage sample is added by letting it flow gently along the walls, followed by the addition of 1 mL of isoamyl alcohol ( $C_5H_{11}OH$ ) to dissolve the fat content. The fat, being less dense, separates and rises to the top of the butyrometer. The neck of the butyrometer is cleaned and sealed tightly. The mixture is then gently agitated until the proteins are fully dissolved by the action of the sulfuric acid—this is evidenced by the complete disappearance of clumps. The butyrometer is inverted repeatedly to aid the mixing; this also leads to a rise in temperature due to the exothermic nature of the reaction. Finally, the butyrometer is placed in a centrifuge for 5 minutes, after which the fat content is read directly from the graduated scale of the butyrometer.(chaibane;2024)

After 5 minutes of hot centrifugation, we immediately proceeded to take the reading:

vertical butyrometer, tip in the air, then take a quick reading on the butyrometer scale

= the value corresponding to the upper level of the fat column

= the value corresponding to the lower level of the fat column

### 3.8. Determination of Fatty Acids by GC/MS :

#### 3.8.1. Principle:

The purpose of this method is to extract and convert the total lipids of a sample in order to perform a fatty acid analysis using gas chromatography (GC). The method allows for the separation and extraction of total lipids, followed by their transformation into methyl esters. These derivatives are then analyzed qualitatively and quantitatively to determine the composition of fatty acids.

#### 3.8.2. Procedure:

##### Lipid Extraction:

1. The freeze-dried sample is placed into a sealed methylation tube.
2. 0.1 g of freeze-dried sample is esterified using metallic KOH in hexane.
3. The mixture is homogenized to ensure effective separation of the components.
4. The solution is then centrifuged at 1100 g for 10 minutes.

5. The upper organic phase is carefully collected and transferred into a new methylation tube.
6. A second extraction is performed on the remaining aqueous phase using 2 mL of hexane to recover any residual lipids.
7. The combined organic phases are washed with 2 mL of NaCl solution (0.9%, v/v) and centrifuged again (3 minutes at 1100 g).
8. The final organic phase is evaporated under a nitrogen stream to remove all solvents completely.

### 3.8.3. Expression of Results:

- The analysis is carried out using gas chromatography coupled with mass spectrometry (GC/MS).
- Helium is used as the carrier gas at a flow rate of 1 mL/min.
- A capillary column SH-5SIL MS (30 m × 0.25 μm) is employed.
- The methyl esters of the fatty acids are identified and quantified by interpreting the chromatogram, which provides both qualitative and quantitative information on the fatty acid composition of the sample.

## 3.9. Determination of Reducing Sugars by HPLC :

### 3.9.1. Principle:

High-Performance Liquid Chromatography (HPLC) is a chemical analysis technique used to separate, identify, and quantify components in a mixture. It is based on the differential distribution of these components between two phases: a liquid mobile phase and a solid stationary phase. Reducing sugars are separated according to their interactions with these phases and can be detected and measured with high precision.

### 3.9.2. Procedure :

- Fill a syringe with the sample to be analyzed. After allowing it to stand for 15 minutes, the concentration of reducing sugars can be determined.
- Insert the syringe into the injection loop (ensure the syringe volume exceeds the loop capacity).
- Fill the injection loop, which has a capacity of 10 μL.
- Turn the trigger to the “inject” position to start the sample analysis.
- Return the trigger to the “load” position once the injection is complete.

### 3.9.3. Expression of Results :

- Mobile phase: A mixture of water, 0.1% formic acid, and methanol is used to facilitate the elution of sugars through the chromatographic column.
- Stationary phase: (The specific column is not mentioned, but it typically consists of a reverse-phase material suitable for polar compound separation.)
- Injection volume: 10  $\mu$ L of the prepared and filtered sample is introduced into the LC-MS system. (Tedjani, 2024)

### 3.10. Starch content :

This application note describes a method for quantifying soluble starch in fresh quinoa milk using a UV-Visible spectrophotometer at a wavelength of 600 nm, based on the protocol of P.Y. Yap, A. The procedure begins with the preparation of a starch standard solution (S1) by dissolving 0.05 grams of starch in 10 ml of double-distilled water, followed by the addition of 40 ml of double-distilled water heated to 90°C to ensure complete solubilization. Simultaneously, the iodine-potassium iodide reagent ( $I_2 + KI$ ) is prepared by dissolving 2.54 grams of iodine ( $I_2$ ) and 3 grams of potassium iodide (KI) in 100 ml of distilled water. This reagent will be used to detect starch through the formation of a characteristic blue complex.

For sample preparation, 1 ml of fresh quinoa milk is diluted with 9 ml of double-distilled water to create Solution A. From this, 1 ml of Solution A is added to 9 ml of double-distilled water to produce Solution B. Then, 1 ml of Solution B is further diluted with 9 ml of double-distilled water to obtain Solution C, the final sample used for spectrophotometric analysis. To quantify the starch, an aliquot of Solution C is mixed with a measured amount of the iodine-potassium iodide reagent, and after color development, the absorbance is measured at 600 nm using a UV-Visible spectrophotometer. The starch concentration in the quinoa milk is determined by comparing the absorbance to a standard curve prepared from known concentrations of the starch standard solution.

### 3.11. Fibre dosage :

The crude fiber content is determined using the Weende method, as adapted by (Van Soest & Robertson; 1979). This technique is based on the removal of non-cellulosic materials through treatment with sulfuric acid and potassium hydroxide solutions.

The procedure begins with the determination of the sample's moisture content by heating it at 105 °C in an oven until a constant weight is achieved, followed by cooling in a desiccator. Approximately 200 mg of quinoa milk powder is used. Up to 150 ml of a 1.25% sulfuric acid solution, preheated on a hot plate, is added to the sample. Three to five drops of n-octanol are introduced as an anti-foaming agent. The mixture is then brought to a boil and maintained at boiling for 30 minutes. After boiling, the acid is removed.

The residue is washed three times with hot distilled water (filling the crucible to the top each time), using compressed air to mix the contents after each wash. Following the final wash, 150 ml of preheated 1.25% potassium hydroxide (KOH) solution and 3 to 5 drops of anti-foam agent are added. The mixture is again boiled for 30 minutes.

After boiling, solid particles are removed, and the residue is washed with hot distilled water. A final rinse with cold distilled water is followed by three washes with 25 ml of acetone, mixing each time using compressed air. The crucibles are then dried in an oven at 105 °C until a constant weight is reached and cooled in a desiccator. The final weight represents the crude fiber content plus ash. The crude fiber content alone is calculated by subtracting the ash weight obtained after incineration (Boual, 2014)

### 3.12. Total sugar dosage :

The total sugar content of the different fractions was determined using the colorimetric method developed by (Dubois & al. 1956). The phenol-sulfuric acid assay is used to quantify total sugars (Dubois & al., 1956). In this method, monosaccharides are dehydrated in the presence of concentrated sulfuric acid to form furfural derivatives. These derivatives react with phenol to form yellow-orange colored complexes. The intensity of this color, which is proportional to the sugar concentration, is measured by assessing the increase in optical density at 490 nm.

To perform the assay, 1 ml of the sample solution and 1 ml of phenol solution are placed in a Pyrex tube. The phenol solution is prepared by dissolving 5 g of phenol in 100 ml of distilled water. After homogenization, 5 ml of concentrated sulfuric acid ( $H_2SO_4$ , 95–98%) is added. The tubes are then incubated in the dark for 30 minutes. Absorbance is measured at 490 nm using a spectrophotometer.

### 3.13. Antioxidant activity :

#### 3.13.1. Determination of Radical Scavenging Activity by DPPH Assay:

The radical scavenging activity of different extracts was determined using the DPPH assay, according to the method described by (Chang *et al*;2001). The decrease in absorbance of the DPPH solution after the addition of an antioxidant was measured at 517 nm. Ascorbic acid (10 mg/mL in DMSO) was used as the positive control.

#### 3.13.2. Principle :

1,1-diphenyl-2-picrylhydrazyl (DPPH) is a stable free radical (appearing as a red-colored powder) that turns yellow when it is neutralized. The DPPH assay exploits this color change to evaluate the free radical scavenging activity of antioxidant compounds or extracts. The reaction between DPPH and an antioxidant (HA) Antioxidants donate hydrogen atoms to DPPH radicals, reducing them to DPPH-H, leading to a decrease in absorbance. The degree of discoloration reflects the radical scavenging potential of the tested compounds.

#### 3.13.3. Preparation of Reagents :

A 0.1 mM DPPH solution was prepared by dissolving 4 mg of DPPH in 100 mL of ethanol.

#### 3.13.4. Procedure :

Different volumes (from 2 to 20  $\mu$ L) of quinoa milk were adjusted to 40  $\mu$ L using DMSO. Then, 2.96 mL of DPPH solution (0.1 mM) was added to each sample. The reaction mixture was incubated in the dark at room temperature for 20 minutes. After incubation, the absorbance was measured at 517 nm. A control (blank) was prepared by mixing 3 mL of DPPH solution without sample.

### 3.14. anti-inflammatory activity :

The in vitro anti-inflammatory activity of the quinoa milk powder was evaluated using the protein denaturation inhibition method. Four types of solutions were prepared for this analysis:

- **The test solution** (0.5 ml) contained 0.45 ml of bovine serum albumin (BSA) at 5% and 0.05 ml of aqueous or methanolic extract at a concentration of 250  $\mu$ g/ml.
- **The control test solution** (0.5 ml) consisted of 0.45 ml of BSA at 5% and 0.05 ml of distilled water.

- **The product control solution** included 0.45 ml of distilled water and 0.05 ml of aqueous extract (250 µg/ml), without BSA.
- **The standard solution** was composed of 0.45 ml of BSA at 5% and 0.05 ml of diclofenac sodium at 250 µg/ml, used as a reference anti-inflammatory drug.

All solutions were adjusted to pH 6.3 using 1N HCl. The samples were then incubated at 37°C for 20 minutes, followed by heating at 57°C for 3 minutes. After cooling, 2.5 ml of phosphate buffer (pH 6.3) was added to each solution. Absorbance was measured at 416 nm using a UV-visible spectrophotometer. The percentage inhibition of protein denaturation was calculated using the following formula:

$$\% \text{ inhibition} = 100 - [(\text{DO of test solution} - \text{OD of control}) / \text{OD of test control}] \times 100.$$

OD: optical density.

where OD represents the optical density. The control test is considered to represent 100% denatured proteins, and the results were compared to those obtained with diclofenac sodium at 250 µg/ml. (Haioun, Hamoudi. 2015).

# **Chapter III:**

## **Result and discussion**

**I. Organoleptics analysis result :****1. Appearance :**

- Smooth, free-flowing consistency.
- No sedimentation or floating particles.
- Homogenized appearance if properly processed.

**2. Color :**

- Uniform off-white to pale beige.
- Slight natural turbidity is acceptable.

**3. Odor (Aroma):**

- Mild, pleasant cereal or nutty scent (typical of quinoa).
- Slight sweetness if date syrup or other natural sweeteners are added.

**4. Taste (Flavor Profile) :**

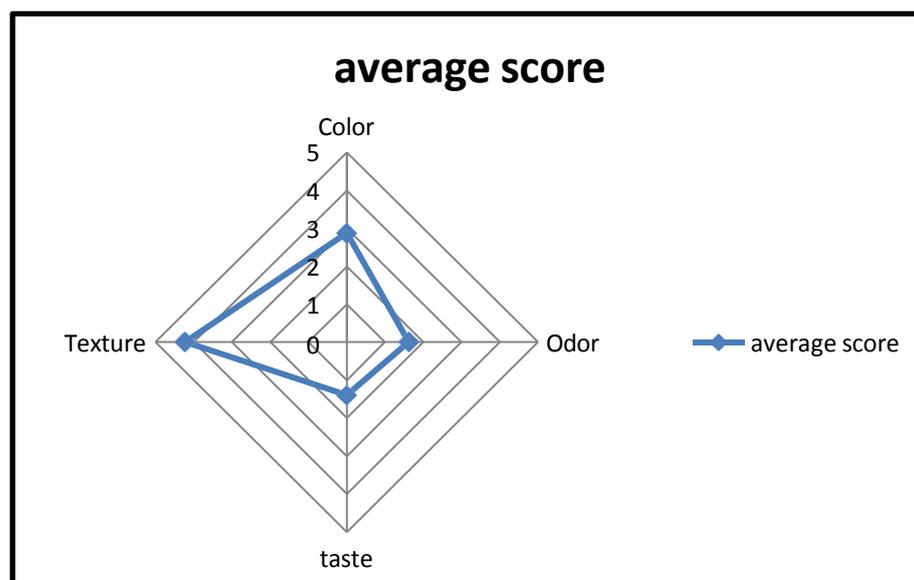
- Lightly nutty or earthy taste, mild and smooth.
- Balanced sweetness (if sweetened).
- Absence of bitterness or astringency.

**5. Texture / Mouthfeel :**

- Smooth and slightly creamy.
- Moderate viscosity
- Minimal graininess

**6. Aftertaste:**

- Light, clean cereal finish.
- Slight nutty sweetness



**Figure 03 : Quinoa milk acceptance rating scale**

The sensory evaluation of quinoa milk reveals heterogeneous consumer attitude towards different attributes. Texture was the highest (4.22/5) and reflects that the product possesses a good mouthfeel, which is due to its colloidal stability and fiber. This is a positive indicator of its probable acceptability in the aspect of stability.

On the other hand, the smell (1.62/5) and flavor (1.39/5) were worst rated, indicating that the panelists were not highly accepting the smell and flavor.

The color fell in the middle band (2.88/5), maybe a reflection of customer unfamiliarity with the appearance of quinoa milk relative to traditional dairy or mass market plant milks like almond or soy.

Overall, the results indicate that although quinoa milk delivers a suitable texture, flavor and odour improvement is essential to an enhanced consumer acceptance. Adjustments like sweeteners, natural flavoring, or the processing technology improvement could improve the sensory quality of the final product.

## II. Physical- chemical characteristics:

### 1. Calculates yeilde :

From 100 g of quinoa, a volume of 1.4 L of quinoa milk was obtained, a yield of 1.4 %, corresponding to 14 ml of milk per gram of seeds. After freeze-drying this milk, 25 g of powder were recovered, which represents an overall yield of 25% compared to the raw material. Furthermore, the dry matter yield of the milk obtained after drying is 1.79%, indicating that 100 ml of quinoa milk gives approximately 1.79 g of powder.

## 2. pH:

The pH of the analyzed quinoa milk is 5.5, which corresponds to moderate acidity. This value is typical of unfermented plant-based beverages and can be influenced by several factors related to the raw material, the manufacturing process, and additions to the formula.

- ❖ Quinoa variety used: Different quinoa varieties have varying chemical compositions, which can lead to slight variations in the final pH of the milk.
- ❖ Processing method: The quinoa milling method and the type of filtration used can influence the pH by altering the extraction of acidic or alkaline compounds.
- ❖ Shelf life: Over time, natural microorganisms can produce lactic acid, leading to a gradual decrease in pH during storage.

## 3. Ash content :

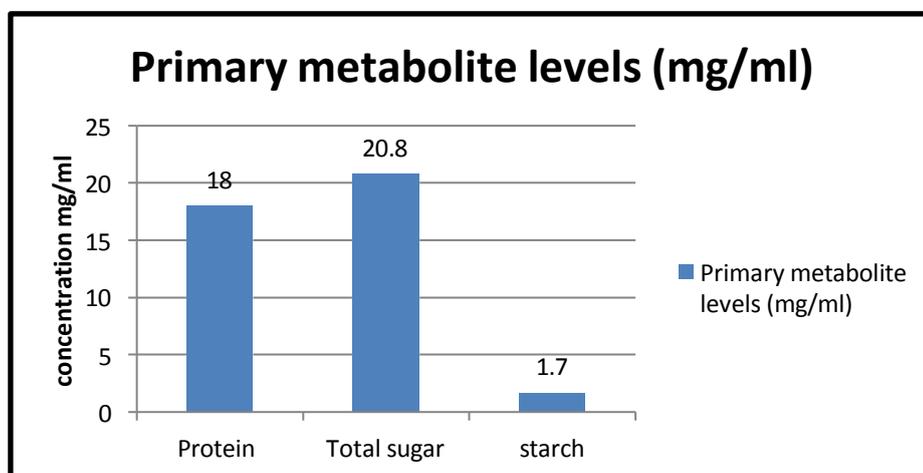
The 1% ash content observed in the quinoa milk indicates a relatively high concentration of minerals. This value exceeds those commonly reported in the literature, where ash contents typically range from 0.22% to 0.52%, depending on the formulation and processing methods used (Jain, 2023). Such a high value suggests a more concentrated formulation or a lower dilution of the milk, which naturally increases the mineral load of the final product. From a nutritional standpoint, an ash content of 1% represents a significant advantage (Gabal, 2020). Ash is a general indicator of the presence of essential minerals such as calcium, phosphorus, iron, and magnesium, which play key roles in bone, muscle, and enzymatic metabolism. Therefore, quinoa milk with this level of ash may be suitable for specific diets, particularly vegetarian, vegan, or therapeutic nutrition plans (Jain, 2023).

## 4. Moisture content:

The results obtained indicate that the dry matter content of quinoa milk powder is 90.5%, corresponding to a moisture content of 9.5%. However, considering the composition of the original liquid drink, water remains the major constituent, representing 57.58% of its content. In addition, the moisture content of quinoa seeds, one of the key raw materials, varies between 5% and 25% (Khan Nadiya Jan *et al.*, 2018). These values highlight the initially high water content of the product before freeze-drying, reflecting the need for moisture reduction to ensure powder stability and shelf life.

### 5. Primary Metabolite Content:

After the calculations, the following results were found in the figure format above:



**Figure04: Primary metabolite contents of quinoa milk.**

The results indicate that quinoa milk contains a significant amount of total sugars (20.8 mg/ml), followed by protein (18 mg/ml) and a lower concentration of starch (1.7 mg/ml). This composition is consistent with that of raw quinoa, recognized for its nutritional richness (Vega-Gálvez & al., 2010).

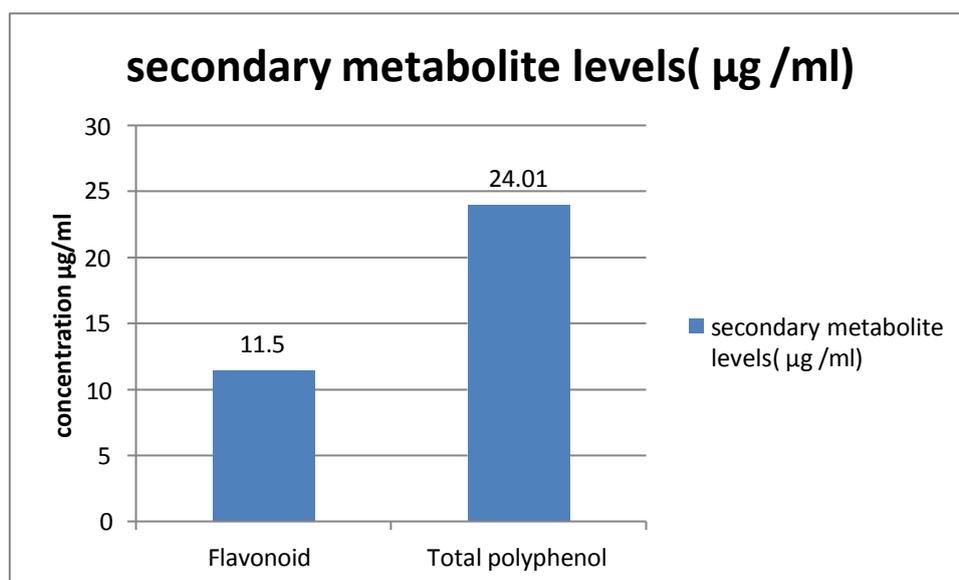
The high carbohydrate content may be due to the presence of simple sugars naturally present in the seeds, but also to enzymatic or thermal transformations during the milk manufacturing process (Ruales & Nair, 1993). This high sugar content improves the product's flavor and may contribute to its sensory acceptability.

Quinoa proteins are particularly interesting because they possess a complete essential amino acid profile, which is rare for a plant-based source (Vega-Gálvez & al., 2010). This quality gives quinoa milk a high nutritional value, useful for vegetarian diets or people with lactose intolerance.

Finally, the low starch content is probably explained by its reduced solubility in water and its partial elimination during the filtration steps. This factor may contribute to the milk's moderate glycemic index, which is favorable for diabetics (Ruales & Nair, 1993).

### 6. Secondary Metabolite Content:

After calculations, the following results were found in the figure format above:



**Figure05 : Secondary metabolite content of quinoa milk**

Quinoa milk also contains significant amounts of flavonoids (11.5 µg/ ml of quinoa) and total polyphenols (24.01 µg/ ml of quinoa). These secondary metabolites play an important role in antioxidant protection and contribute to the product's functional value. Flavonoids, such as quercetin and kaempferol, identified in quinoa seeds, are known for their anti-inflammatory, antiviral, and cardioprotective effects (Tang & al., 2015). Their presence in milk suggests that some of these properties are retained after processing, making it attractive as a functional food.

Similarly, the presence of polyphenols helps limit lipid oxidation in the product, thus improving its shelf life and sensory properties (Gawlik-Dziki & al., 2013). Although their concentration is lower than that of flavonoids, their synergistic action enhances the product's overall antioxidant potential. These results are consistent with other studies showing that quinoa, even when processed, retains a significant portion of its bioactive compounds (Repo-Carrasco-Valencia & Serna, 2011), thus reinforcing its reputation as a "health food".

### 7. Fiber Content:

The quinoa milk analyzed had a dietary fiber content of 17% by dry weight, which is remarkable for a plant-based beverage. This value reflects the richness of soluble and insoluble fiber naturally present in quinoa seeds, which is partially transferred into the milk during the extraction process (Repo-Carrasco-Valencia & Serna, 2011).

Dietary fiber plays a key role in regulating intestinal transit, reducing cholesterol

absorption, and modulating blood sugar levels after meals (Vega-Gálvez & *al.*, 2010). A content of 17% places this quinoa milk among the plant-based beverages with the highest fiber content, which could make it a particularly attractive product for consumers seeking digestive and metabolic health benefits.

Furthermore, fiber acts as a prebiotic, promoting the growth of beneficial intestinal bacteria. This gives the product significant functional potential, particularly in diets targeting gut health and immunity.

It should be noted that the fiber content can vary depending on the manufacturing process (milling, filtration, concentration) and the quinoa variety used. The retention of such a high proportion of fiber in the final product demonstrates that the extraction process used here is relatively effective in preserving the structural components of the grain.

#### 8. Fat content :

The fat content measured in this quinoa sample is 1%. which reflects a relatively low lipid composition compared to other milks of animal origin, rate corresponds to the fat content rate of quinoa milk is 0.2 g in 100 ml ([mynetdiary.com](http://mynetdiary.com))

#### 9. Determination of reducing sugars:

Chromatographic analysis performed by HPLC reveals a diverse carbohydrate composition in quinoa milk, with a marked predominance of simple sugars and disaccharides. The identified peaks correspond to:

- **Fructose** (Ret. Time 2.050 min): 1,605,987 area units, or 23.21% of the relative content
- **Glucose** (3.066 min): 227,301 units, or 3.29%
- **Sucrose** (3.641 min): 2,265,412 units, or 32.74%

The high proportion of sucrose is typical of aqueous quinoa extracts, Fructose, naturally present in seeds has a low glycemic index, which can be beneficial for diabetics in small amounts. However, its high presence (23%) requires moderation in sensitive individuals. Glucose, although poorly represented here (3.29%), plays an immediate role in the body's energy response. Its low concentration can be an advantage in formulations aimed at reducing the overall glycemic load of milk.

#### 10. Fatty acid content :

According to the results obtained by the GC/MS, quinoa milk has an interesting fatty acid profile, influenced by the composition of a main ingredient: quinoa milk. The

sample is rich in unsaturated fatty acids of the omega-9 and mega-6 groups;

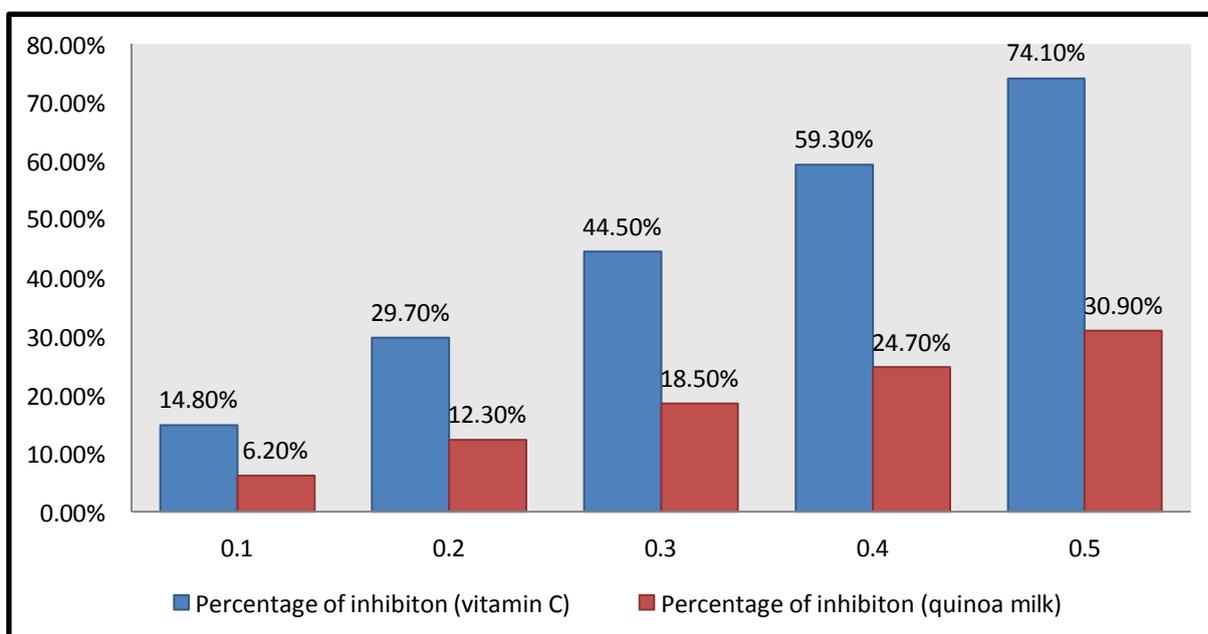
- 12-Octadecadienoic acid (Z,Z)-, methyl ester
- 9-Octadecenoic acid, methyl ester
- cis-13-Eicosenoic acid, methyl ester
- 9-Octadecenoic acid (Z)-, methyl ester

The lipid profile of quinoa milk reveals a richness in unsaturated fatty acids, notably linoleic acid (omega-6), oleic acid (omega-9), and gondoic acid. These compounds, identified as methyl esters, give quinoa milk a high nutritional value. Linoleic acid is essential for the body, while oleic acid is beneficial for cardiovascular health. The presence of these fatty acids makes quinoa milk a healthy and functional plant-based alternative, particularly suitable for vegetarian and vegan diets.

## 11. Antioxidant Activity:

### 11.1. DPPH:

The percentage inhibition of DPPH cation radicals by quinoa milk and ascorbic acid was determined, and the results are presented in Figure



**Figure 06 : Percentage of inhibiton of ascorbic acid and quinoa milk**

	Ascorbic acid	Quinoa milk
<b>Percentage of inhibition</b>	74.10±0.23%	30.90±0.09%

**Table 09: Percentage of inhibition of ascorbic acid and quinoa milk**

The results presented show higher activity for vitamin C (74.10±0.23%) compared to quinoa milk (30.90±0.09%). This observation is consistent with numerous studies showing that vitamin C (ascorbic acid) is a powerful antioxidant capable of effectively neutralizing free radicals (Carr & Frei, 1999). It acts as an enzyme cofactor and plays an essential role in cellular protection against oxidative stress (Padayatty & al., 2003). In comparison, quinoa milk, although less active, still exhibits significant antioxidant activity. This activity can be attributed to quinoa's richness in phenolic compounds, flavonoids, as well as essential vitamins and amino acids (Tang & al., 2015). Several studies have demonstrated that quinoa extracts possess significant antioxidant and anti-inflammatory properties, although less so compared to pure antioxidants such as vitamin C (Repo-Carrasco-Valencia & al., 2010).

It should also be noted that differences in activity may result not only from the nature of the compounds, but also from the extraction methods, concentrations used, and experimental conditions (Kähkönen & al., 1999). Thus, the potential of quinoa milk could be enhanced by enriching it or combining it with other bioactive substances.

It should also be noted that ascorbic acid is more effective; IC<sub>50</sub> values were calculated and the results are shown in Table 6

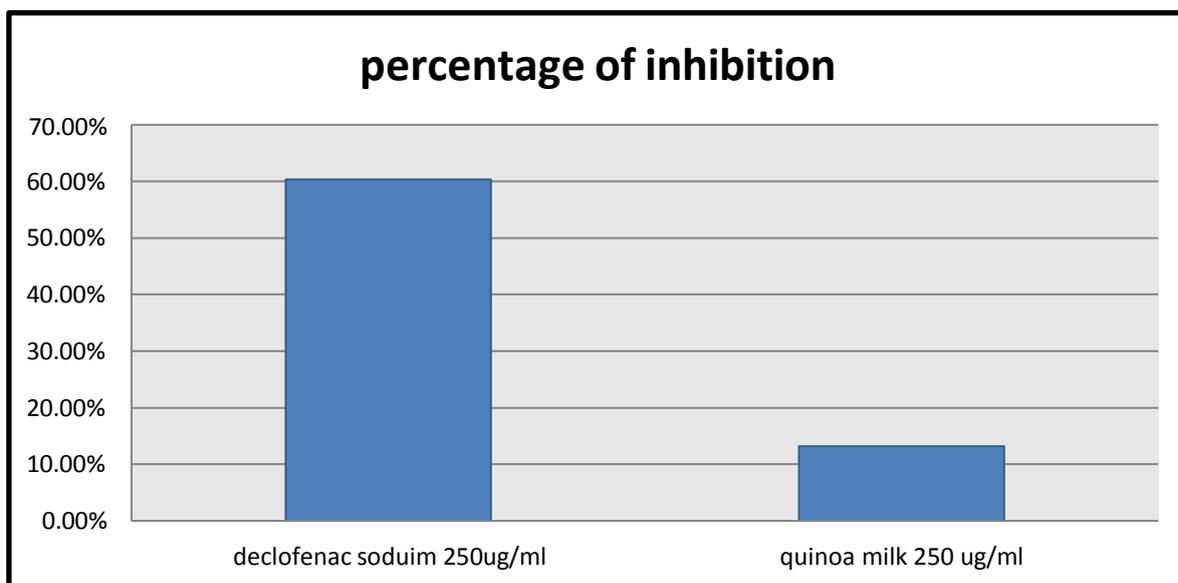
**Table 10 : IC<sub>50</sub> of ascorbic acid and quinoa milk**

	Vitamin C	Quinoa milk
<b>IC 50</b>	0.324±0.14mg/ml	0.9±0.24 mg/ml

It appears that the antioxidant power of quinoa milk is significantly lower than that of ascorbic acid, where the IC<sub>50</sub> of quinoa milk is 0.9±0.24 mg/ml, while that of ascorbic acid is around 0.324±0.14 mg/ml .

## 12. Anti-inflammatory activity:

The table shows the results of the in vitro anti-inflammatory activity of quinoa milk, which consists of evaluating the percentages of inhibition of the denaturation of Bovine Serum Albumin (BSA).



**Figure 07 : Percentage of inhibition of denaturation of BSA denaturation at the concentration of 250 µg/ml**

	Diclofenac soduim	Quinoa milk
<b>% inhibition of denaturation</b>	60.35±0.12%	13.18±0.01%

**Table 11 : Percentage of inhibition of denaturation of BSA at the concentration of 250 µg/ml**

The results obtained indicate that diclofenac sodium exhibited significantly higher inhibition (60.35±0.12%) compared to quinoa milk (13.18±0.01%). These data corroborate previous research demonstrating the anti-inflammatory efficacy of diclofenac sodium, a well-established nonsteroidal anti-inflammatory drug (NSAID) that acts by inhibiting cyclooxygenase enzymes (COX-1 and COX-2), responsible for the synthesis of pro-inflammatory prostaglandins (Zarghi & Arfaei, 2011).

In contrast, although the percentage of inhibition in quinoa milk was lower, it remains significant, suggesting the presence of bioactive compounds with anti-inflammatory potential. Studies have reported that quinoa contains flavonoids, saponins, and phenolic acids with antioxidant and anti-inflammatory activity (Tang & al., 2015). These compounds may modulate the inflammatory response through indirect mechanisms,

such as reducing oxidative stress or inhibiting pro-inflammatory mediators such as TNF- $\alpha$  or IL-6 (Pasko *&al.*, 2009).

Thus, although the efficacy of quinoa milk is lower than that of diclofenac, its effect remains significant and could be leveraged in natural or complementary formulations for the management of inflammation, particularly in subjects sensitive to the side effects of NSAIDs. Further studies are nevertheless needed to isolate the active ingredients responsible and elucidate their mechanisms of action.

# Conclusion

### Conclusion:

The study was interested in the production, characterization, and evaluation of a quinoa (*Chenopodium quinoa Willd.*) vegetable beverage with the objective of providing a nutritionally adequate and functionally healthy substitute for conventional dairy products. The product was designed to meet the requirements of the lactose intolerant, diabetic, and the vegetarian and vegan population.

The physicochemical analysis of the quinoa drink revealed that it is rich in essential nutrients. It contains complete proteins, that is, it provides all nine essential amino acids, a feature not usual in plant foods. The beverage is also rich in dietary fiber, which promotes digestion and stabilizes blood glucose levels. It also contains a high degree of flavonoids and polyphenols—naturally occurring compounds with potent antioxidant activity. It contains high unsaturated fats, predominantly linoleic and oleic acids, which are recognized to be beneficial to the cardiovascular system. Reducing sugars were also detected, which are accountable for the beverage's minimal sweetness and flavor complexity.

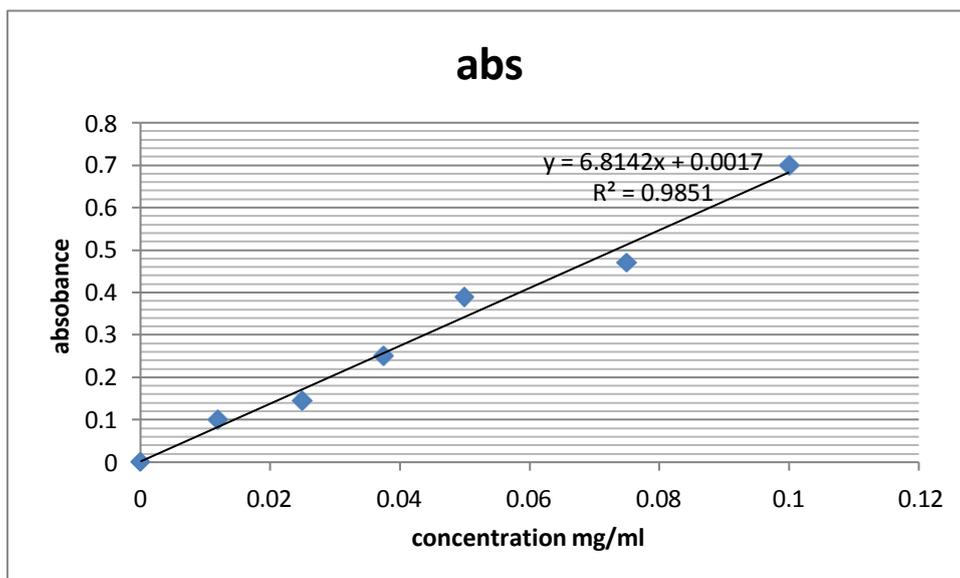
The biological activities of the milk were established by *in vitro* tests for antioxidant and anti-inflammatory activities. Although the antioxidant activity was less than that of ascorbic acid (vitamin C), it was good nonetheless due in large measure to the polyphenol content. Similarly, the anti-inflammatory activity, although less potent than that of diclofenac sodium (a reference pharmaceutical anti-inflammatory drug), was nonetheless significant. These findings point to the drink's potential as a functional food that can have a role in the prevention of oxidative stress and inflammation-based diseases.

To ascertain consumer acceptability, sensory analysis was done for color, odor, taste, and texture. It was noted from the results that the consumers liked the product overall. While taste and odor had lower scores—possibly due to quinoa's natural bitterness texture was particularly favored, suggesting a smooth and pleasant mouthfeel. This means that the drink, as formulated, already has a high likelihood of consumer acceptance, but that subsequent adjustments in taste and aroma can render it even more appealing.

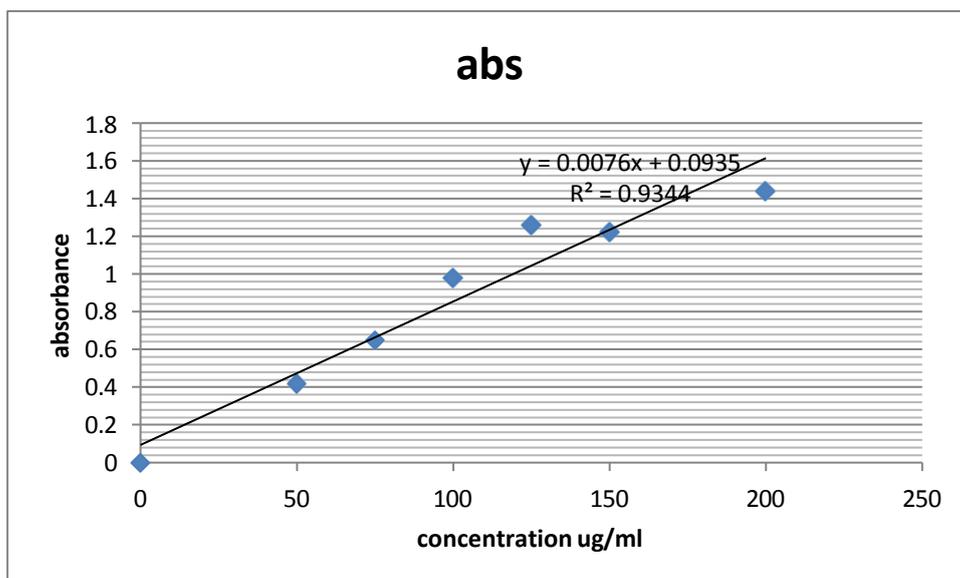
In final, this study demonstrates that a quinoa milk is not only possible to

formulate, but also has great nutritional and functional value. That it is high in essential nutrients and bioactive molecules makes it well-placed as an adequate alternative to animal milk. Yet, further research is recommended in this regard to optimize the sensory attributes of the beverage, its shelf life and stability, and to study its effects through in vivo research. These efforts can further strengthen its position as a potential functional food in the plant-based beverage sector.

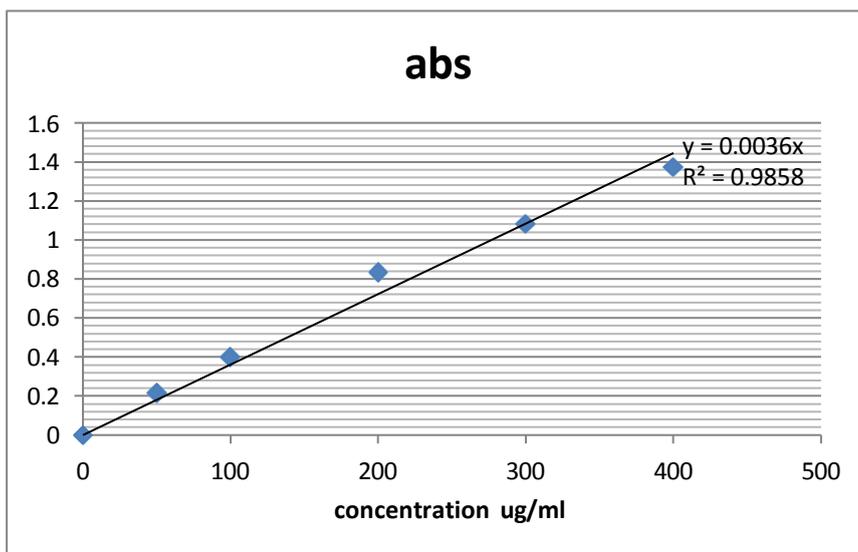
# Annexes



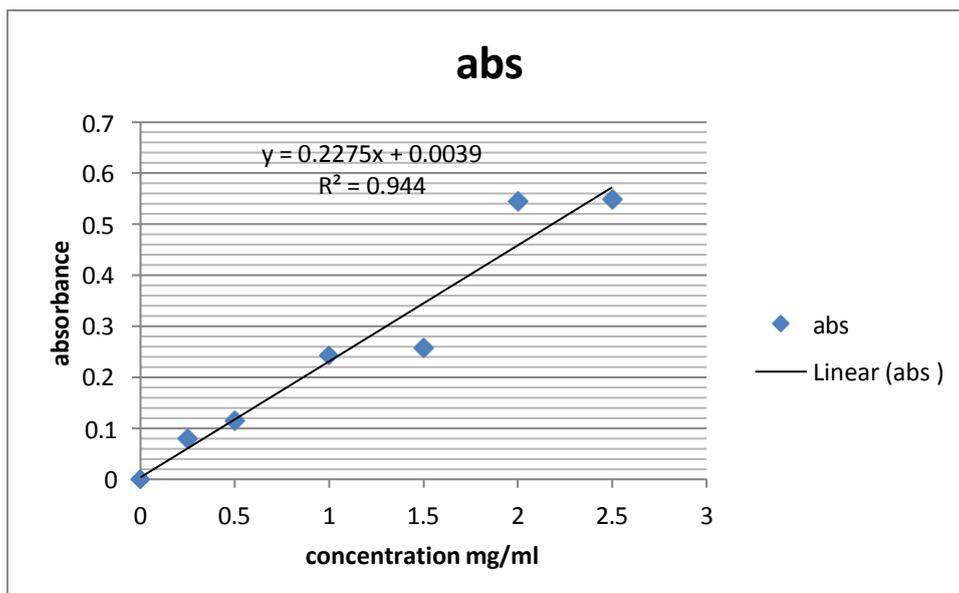
Annex 1 : calibration curve of glucose



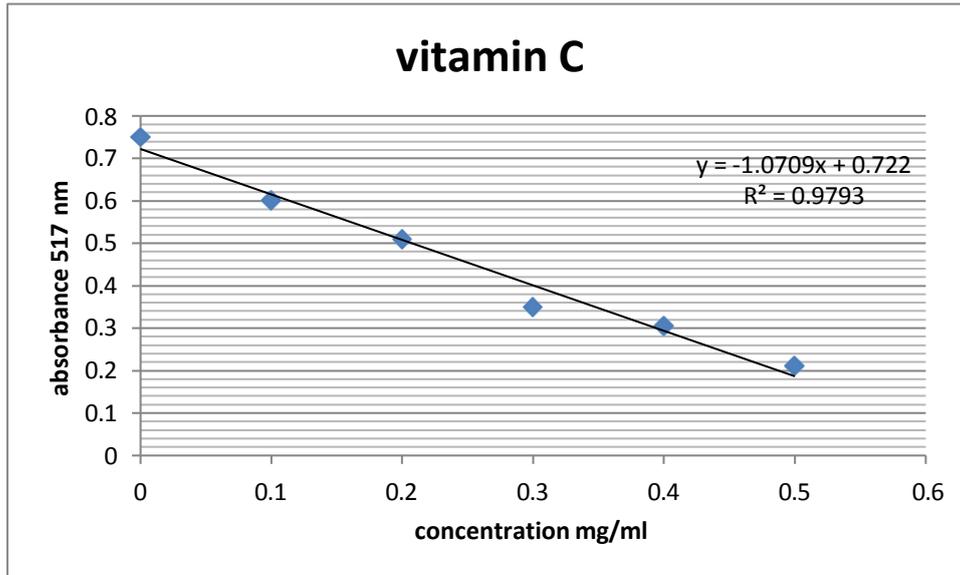
Annex 2: calibration curve of gallic acide



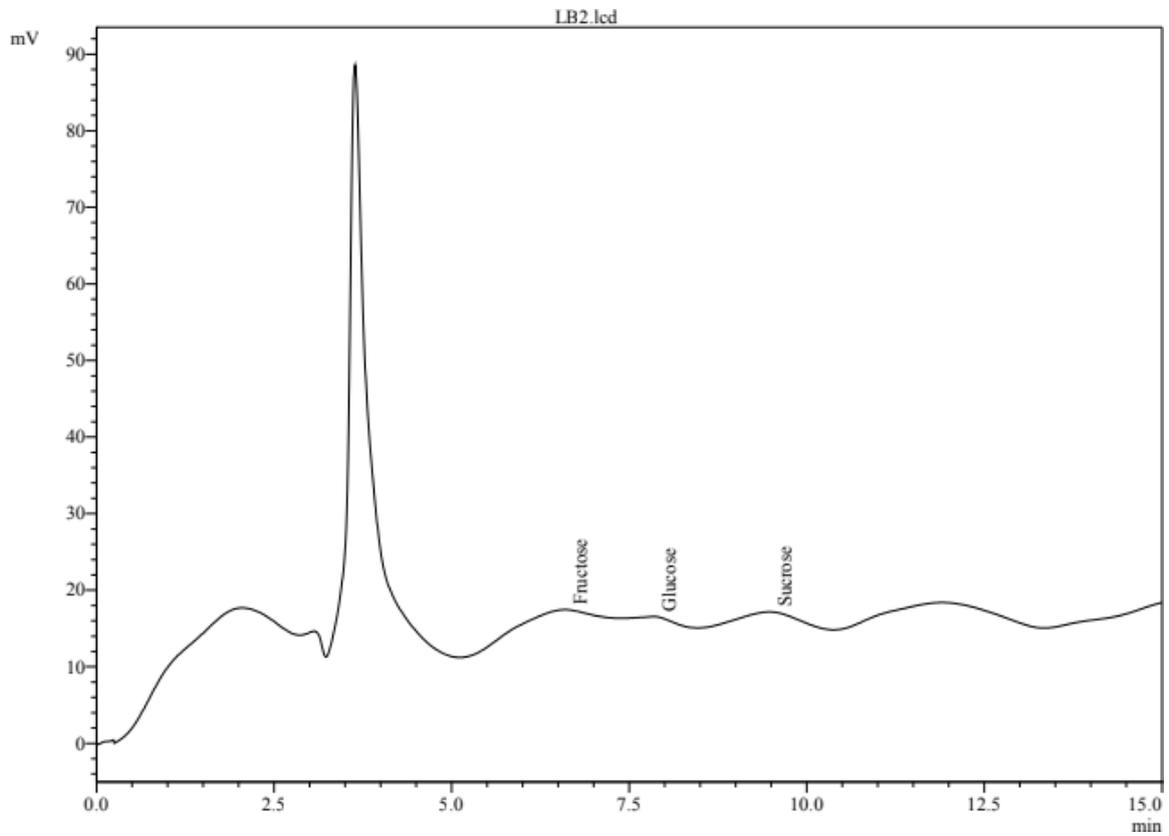
Annex 3 : calibration curve of quercetin



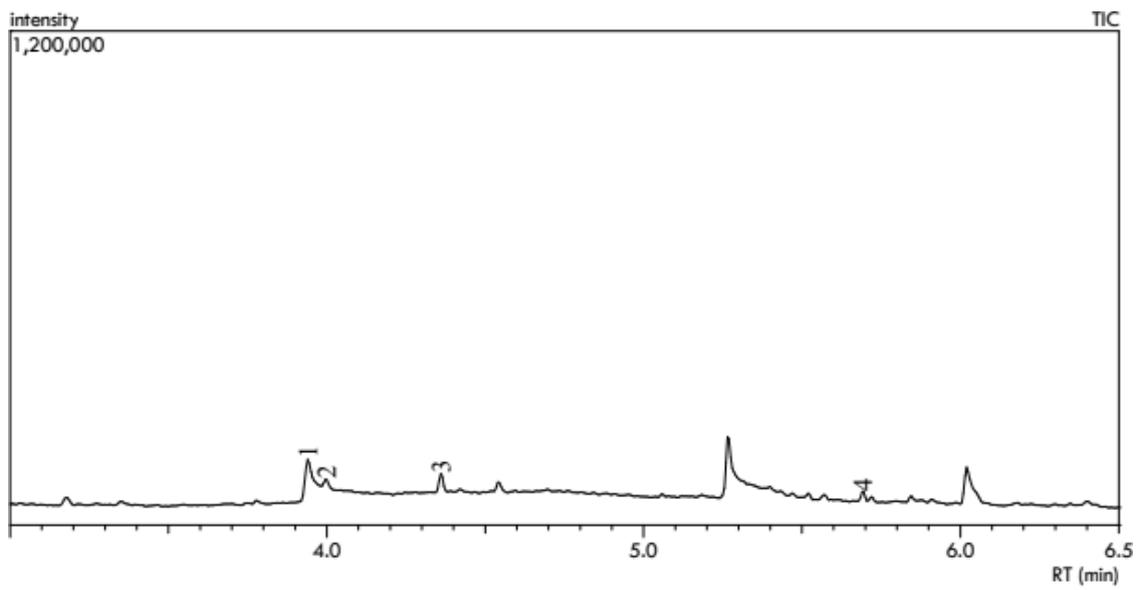
Annex 4 : calibration curve of starch



**Annex 05 : calibration cuve of ascorbic acid**



**Annex 6 : result of redactor sugar of quinoa milk with HPLC**



**Annex 7 : result of fatty acids measured by GC/MS**



**Annex 08 : hot centrifuges**



**Annex 09 : HPLC**



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# Résumé

### Résumé

Ce travail de recherche porte sur la caractérisation physico-chimique et l'évaluation de la valeur nutritionnelle du lait de quinoa en poudre, en tant qu'alternative végétale au lait d'origine animale. Face à la montée des allergies au lactose, de l'intolérance au gluten et des choix alimentaires végétaliens, le lait de quinoa représente une solution prometteuse grâce à sa richesse en protéines, fibres, acides aminés essentiels, vitamines et minéraux.

La méthodologie adoptée comprend la préparation d'une boisson végétale à base de quinoa, suivie de sa déshydratation pour obtenir une poudre de lait. Des analyses approfondies ont été menées afin d'évaluer ses propriétés physico-chimiques (pH, humidité, cendres, matières grasses, sucres, fibres), sa composition en métabolites primaires et secondaires (protéines, flavonoïdes, polyphénols), ainsi que son profil en acides gras. L'activité antioxydante et anti-inflammatoire du produit final a également été évaluée.

Les résultats obtenus montrent que la poudre de lait de quinoa possède un profil nutritionnel équilibré, une teneur élevée en composés bioactifs, et présente une activité antioxydante et anti-inflammatoire notable. Ces qualités font de ce produit un candidat pertinent pour une consommation fonctionnelle, notamment pour les personnes souffrant d'intolérances alimentaires ou suivant un régime alimentaire sain.

**Mots-clés** : Quinoa, lait végétal, poudre de lait, analyse physico-chimique, nutrition, antioxydants.

### Abstract

This research focuses on the physicochemical characterization and nutritional evaluation of quinoa milk powder as a plant-based alternative to animal-derived milk. With the increasing prevalence of lactose intolerance, gluten sensitivity, and vegan dietary choices, quinoa milk offers a promising solution due to its richness in proteins, fibers, essential amino acids, vitamins, and minerals.

The study methodology involved the preparation of a quinoa-based plant milk, followed by dehydration to obtain a powdered form. Comprehensive analyses were conducted to assess its physicochemical properties (pH, moisture, ash, fat, sugars, fibers), its content of primary and secondary metabolites (proteins, flavonoids, polyphenols), and its profile of fatty acids. The antioxidant and anti-inflammatory activities of the final product were also evaluated.

The results demonstrate that quinoa milk powder has a balanced nutritional profile, a high content of bioactive compounds, and notable antioxidant( and anti-inflammatory activity. These qualities make it a relevant candidate for functional consumption, particularly for individuals with food intolerances or those seeking a healthy diet.

**Keywords:** Quinoa, plant-based milk, milk powder, protein , nutrition, antioxidants,

### ملخص :

يركز هذا البحث على التوصيف الفيزيائي والكيميائي والتقييم الغذائي لمسحوق حليب الكينوا كبديل نباتي للحليب الحيواني. مع تزايد انتشار عدم تحمل اللاكتوز، وحساسية الغلوتين، والخيارات الغذائية النباتية، يُقدم حليب الكينوا حلاً واعدًا لغناه بالبروتينات والألياف والأحماض الأمينية الأساسية والفيتامينات والمعادن.

اشتملت منهجية الدراسة على تحضير حليب نباتي من الكينوا، ثم تجفيفه للحصول على مسحوق. أُجريت تحاليل شاملة لتقييم خصائصه الفيزيائية والكيميائية (الرقم الهيدروجيني، الرطوبة، الرماد، الدهون، السكريات، الألياف)، ومحتواه من المستقلبات الأولية والثانوية (البروتينات، الفلافونويدات، البوليفينولات)، ومحتوى الأحماض الدهنية. كما تم تقييم الأنشطة المضادة للأكسدة والالتهابات للمنتج النهائي. أظهرت النتائج أن مسحوق حليب الكينوا يتميز بمحتوى غذائي متوازن، ومحتوى عالٍ من المركبات النشطة بيولوجيًا، ونشاط مضاد للأكسدة ومضاد للالتهابات ملحوظ. هذه الصفات تجعله مرشحًا مناسبًا للاستهلاك الوظيفي، وخاصةً للأفراد الذين يعانون من عدم تحمل الطعام أو أولئك الذين يسعون إلى اتباع نظام غذائي صحي.

**الكلمات المفتاحية:** الكينوا، حليب نباتي، مسحوق حليب، بروتين، تغذية، مضادات الأكسدة.