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Recycling organic waste: enriching fruity beverages

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Dedication

With deep gratitude and sincere words, this modest work is dedicated to those who, whatever the terms embraced, I could never express to them my sincere love.

To my dear dad for his love and support

You are always in my eyes like a king. Thank you for giving me love, support, motivation and learned me the sense of work and responsibility.

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Acronyms

ABTS : 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid.

AlCl₃ : Aluminium chloride.

α -la : Alpha lactoalbumin.

BSA : Bovine serum albumin.

β -lg : Beta lactoglobulin.

C : Carbon atom.

Ca⁺ : Calcium ion.

CMC : Carboxymethyl cellulose.

WHC : Water holding capacity.

OHC : Oil holding capacity.

DPPH : 1,1-diphenyl-2-picrylhydrazyl.

SFE : Supercritical fluid extraction.

FeCl₃ : Ferric chloride.

Fer (II) : Ferrous iron.

Fer (III) : Ferric iron.

FRAP : Ferric reducing antioxidant power.

g : Gram.

HCl : Hydrochloric acid.

IC50 : 50% inhibitory concentration.

kDa : Kilodalton.

L : Liter.

M : Molar mass.

MAE : Microwave-assisted extraction.

mg/mL : Miligram per milliliter.

Min : Minute.

mM : Millimolar.

NaCl : Sodium chloride.

NaOH : Sodium hydroxide.

nm : Nanometer.

Na₂HPO₄ : Hydrogénophosphate de sodium.

NaH₂PO₄ : Sodium dihydrogen phosphate.

-OH : Groupe hydroxyle.

pH : Hydrogen potential.

p/v : Weight by volume.

TFC : Total flavonoid content.

TPC : Total polyphenol content.

Uv-vis : Ultraviolet-visible.

UAE : Ultrasound-assisted extraction.

µg GAE/mg : Microgram of gallic acid equivalents per milligram.

µg QE/mg : Microgram quercetin equivalents per milligram.

µL : Microliter.

% : Percentage.

°C : Degrees Celsius.

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General introduction

General introduction

Worldwide, the agri-food sector generates large quantities of solid and liquid waste. Annual food production is around 1.3 billion tones. Different industries contribute differently to food waste: the beverage industry accounts for 26%, followed by the dairy and ice cream industry with 21.3%. Fruits and vegetables manufacturing and storage contribute 14.8%, while cereal and starch products account for 12.9%. The meat products industry discharges 8% of waste, vegetable and animal oils and fats 3.9%, and fish. Waste from the manufacture of other food products accounts for 12.7% (**Baiano, 2014**).

The disposal of industrial waste, including by-products, contributes significantly to various forms of pollution, such as contamination of soil, air and water, as well as food sources. However, in the face of these environmental challenges, it is essential to recognize that industrial by-products also contain valuable bioactive elements. These include phenolic compounds, flavonoids, carotenoids and proteins, which offer considerable potential for the development of new products and applications (**Berger, 2003; Baiano, 2014**).

The bioactive molecules present in the foods we eat have several beneficial implications from various points of view. Environmentally, they reduce the creation of pollution sources. Economically, they minimize the costs associated with waste handling and management. Biologically, their antioxidant properties prevent the formation of free radicals. Nutritionally, they improve organoleptic quality, including color, taste, odor and flavor. Furthermore, they play a crucial role in improving human well-being and preventing various illnesses such as diabetes and cardiovascular disease (**Melini et al., 2020; Squillaci et al., 2021; Valencia-Hernandez et al., 2021; Liu et al., 2023**).

The aim of food enrichment is to increase the levels of micronutrients in foods that have been identified as deficient, in order to promote their consumption. It is essential that the enrichment process is simple and results in fortified foods that are not only safe, but also acceptable to consumers in terms of taste, texture, aroma and affordability. The degree of enrichment is determined by the specific nutritional needs of the target demographic groups and their consumption habits (**Berger, 2003; Squillaci et al., 2021; Valencia-Hernandez et al., 2021**).

Our study focuses on enriching RAMY juice by exploiting organic waste, such as juice residues rich in phenolic compounds and fermented milk waste rich in protein. This innovative approach aims to valorize these wastes as valuable components for RAMY juice, while reducing food waste and creating value-added juices.

Within the framework of this study, this manuscript is structured in three main parts:

The first part consists of an in-depth review of the literature on waste recovery, focusing on the definition of waste and a detailed analysis of RAMY juice.

The second part presents our experimental approach, including the protocols used. This section aims to achieve several objectives, namely :

- The extraction of phenolic compounds and proteins from juice waste and fermented milk waste.
- Quantification of phenolic and flavonoid compounds and protein content of waste extracts.
- Evaluation of the antioxidant activity of the obtained extracts.
- Enrichment of RAMY juice with selected waste extracts.

Finally, the third section presents the results of our study, including extraction yields, polyphenolic and protein content, and antioxidant activity of the extracts. An in-depth comparison will also be made between unenriched and enriched RAMY juices.

Part I : Literature review

I. Waste and waste recovery

I.1. Waste

I.1.1. Definition

According to the Algerian Official Gazette and the law of December 15, 2001, waste is legally defined as any residual substance resulting from manufacturing, treatment or use procedures, or any other material intended for disposal, planned for disposal, or which the owner or holder is required to dispose of. This comprehensive definition encompasses various forms of waste generated by the industrial, commercial and domestic sectors, and highlights the legal framework designed to regulate waste management practices (**Aliouche et al., 2017**).

I.1.2. Waste classification

A multitude of wastes can be categorized according to their origin and composition, providing a global view of their presence in various activities (**Hou, 2016**).

I.1.2.1. According to origin

Three main categories can be distinguished according to origin:

1. Local authority waste

This type of waste is produced by municipal authorities and includes road debris, market residues, sewage sludge from treatment plants and green waste (**René, 2009**).

2. Household waste

This waste can be classified into two sub-types : bulky and green waste, and household waste (**René, 2009**). All waste in this category is the responsibility of the local authority (**Hou, 2016**).

3. Corporate waste

The industrial, commercial, craft and transport sectors generate approximately ninety million tonnes of waste. This production includes both hazardous and non-hazardous waste, including that generated by the agri-food industry (**René, 2009**).

I.1.2.2. According to nature

According to their nature, five main classes have been identified:

1. Inert waste

These wastes retain their physical, chemical and biological properties unchanged over time and are mainly generated by construction sites and specific industrial sectors (**René, 2009**).

2. Organic waste

Waste can be classified as biodegradable when it consists of organic substances capable of decomposing and degrading naturally over time. This type of waste, often referred to as biowaste, undergoes a process known as biodegradation. During this process, the materials decompose and transform, eventually becoming part of the biosphere. Common sources of biodegradable waste include food residues and other organic materials (**René, 2009**).

3. Common waste

This waste, which is mainly produced by local authorities, can be treated by various methods, such as recycling, composting or incineration (**René, 2009**).

4. Toxic or hazardous waste

This category includes waste mainly from industrial activities, but also from small businesses and institutions, as well as from the agricultural sector (e.g. plant protection products). These wastes may contain varying concentrations of toxic or hazardous substances, requiring special precautions to be taken when handling them. Because of their proven toxicity, many such products are regulated and subject to controls to ensure their safe management and disposal.

Most hazardous industrial waste is transported to specialized collective centers, as advanced treatment techniques are required. They are then stored in one of fifteen hazardous waste storage facilities (**Hou, 2016**).

5. Ultimate waste

Ultimate waste is defined as waste which, whether or not it has undergone a treatment process, can no longer be treated using current technical and economic methods. This includes

waste that cannot be recovered, or whose pollution or hazardous properties cannot be mitigated. (René, 2009; Hou, 2016).

I.2. Food industry waste

Agro-industrial wastes, including those from the agri-food sector, encompass by-products and residues from agricultural and food manufacturing activities. These wastes are nutrient-rich, such as rice straw, husks, corn cobs, sugarcane bagasse and oilseed cakes, rich in complex carbohydrates, proteins, lipids, vitamins, minerals and fibers (Mahongnao et al., 2023; Naik et al., 2023). Agricultural industries generate large quantities of residues every year, whose inadequate management can lead to environmental pollution and risks to human and animal health. Often under-utilized, this waste is burned, landfilled or buried, contributing to greenhouse gas emissions and climate change. However, converting this waste into high-value-added products can not only reduce the expense of disposal, but also help to reduce pollution and promote a sustainable and circular economy (Sadh et al., 2018; Socas-Rodríguez et al., 2021; Mahongnao et al., 2023; Naik et al., 2023).

Agri-food waste, including by-products from fruit, vegetables, dairy products, meat and fish, contains a multitude of bioactive compounds, such as polyphenols, flavonoids, vitamins and proteins (Vilas-Boas et al., 2021). Often overlooked in waste management strategies, these compounds possess antioxidant, antimicrobial and anti-inflammatory properties, making them valuable for food and pharmaceutical applications. By extracting these bioactive compounds from agri-food waste, industries can create value-added products with enhanced nutritional profiles and functional properties (Socas-Rodríguez et al., 2021; Vilas-Boas et al., 2021).

I.3. Waste recovery

The concept of recovery involves transforming waste into valuable products (Nzihou, 2010). It encompasses a set of biotechnological processes that offer an optimal solution by producing high value-added substances (Acourene et al., 2008).

I.3.1. General waste recovery strategies

According to the Algerian Official Gazette, recovery can be described as “all operations involving the reuse, recycling or composting of waste”. There are three main types of recovery :

1. Recycling

Recycling is the process that attracts the most attention in modern waste management strategies. It not only conserves valuable resources, but also contributes to the growth of local economies by creating employment opportunities (**Damien, 2004**).

Article L541-1-1 of the French Environment Code defines recycling as “the process of recovering waste, including organic waste, and transforming it into substances, materials or products intended for their original purpose or for other uses”.

2. Re-employment

Re-employment means using objects that are still usable without changing their original function, thus preventing them from becoming waste (**Desqueyroux and André, 2018**).

3. Reuse

Reuse refers to the process of redirecting waste from its original purpose, enabling it to be reused. This process not only reduces waste, but also optimizes the usefulness of resources by discovering new applications for discarded items. (**Desqueyroux and André, 2018**).

I.3.2. Agri-food waste valorization

The valorization of agri-food industry waste consists of several techniques that extract valuable components from waste streams to produce new products or generate energy. Among the techniques frequently employed for the upgrading of agri-food waste are:

1. Composting

Agri-food waste, such as fruit and vegetable waste, coffee grounds and crop residues, can be composted to create nutrient-rich soil improvers. Composting is a natural process in which micro-organisms break down organic matter to create compost. This compost can improve soil fertility and structure when used on agricultural land (**Liu et al., 2023**).

2. Anaerobic digestion

Organic waste from the food industry, such as crop residues, food scraps and animal manure, can be anaerobically digested to produce biogas (methane and carbon dioxide) and digestate. The biogas can be used as a renewable energy source for power generation or heating, while the digestate can be used as a nutrient-rich fertilizer for agriculture (**Liu et al., 2023**).

3. Animal feed production

Some agri-food wastes, such as spent grains from breweries, fruit and vegetable pulps from juice production and by-products from grain milling, can be reused for animal feed. These wastes are rich in nutrients and can provide a sustainable source of feed for livestock and poultry, reducing dependence on conventional feed ingredients (**Nath et al., 2023**).

4. Biorefinery

Biorefineries use a variety of biomass feedstocks, including agri-food waste, to produce a range of high-value-added products, including biofuels, biochemicals and biomaterials. Through processes such as fermentation, enzymatic hydrolysis and thermochemical conversion, biorefineries transform waste into biobased products, contributing to the development of a circular economy (**Liu et al., 2023**).

5. Extraction of bioactive compounds

Agri-food waste, such as fruit peels, vegetable trimmings and by-products from dairy and meat processing, contain bioactive compounds such as polyphenols, flavonoids and vitamins. For the extraction and recovery of these compounds for use in food additives, supplements and nutraceuticals, various methods are used such as solvent extraction, microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE) and supercritical fluid extraction (SFE) (**Liu et al., 2023**).

II. Fruit juice production

Fruit juice is a liquid extracted from fresh, ripe, healthy fruit, either in its natural state or preserved under appropriate conditions. It is unfermented, but may undergo fermentation (**Díaz-García et al., 2013**).



Figure 1: RAMY MILK fruit juice.

II.1. The composition of fruit juices

II.1.1. Fruit concentrate

Fruit concentrate is defined in the same way as fruit juice, except that it differs in that it contains less water, which results in a higher sugar content. Fruit concentrates may contain volatile aromatic elements, provided these substances are obtained by appropriate physical methods and come from the same variety of fruit (Alimentarius, 2022).

II.1.2. Treated water

Water is a crucial element in the production of food products. For its use to be authorized, it must undergo rigorous treatment by the producing company in order to guarantee its safety and quality (Krasnova, 2018).

II.1.3. Food additives

A food additive is defined as any substance that is not usually consumed as a stand-alone food. Additives are useful for ready-to-eat preparations and are not commonly used as key ingredients in foods, whatever their nutritional value. These substances are added in small quantities to foods during preparation, for specific purposes such as preservation, coloring or sweetening (de Reynal, 2009).

II.1.4. Carboxymethylcellulose (CMC)

CMC powder comes in granular or fibrous form and is either white, slightly yellowish or grayish. This emulsifier has a slight moisture absorption capacity, is odorless and has no perceptible taste (Mondal et al., 2015).

II.2. The fruit juice production process

The production of fruit juice requires a series of steps and precise quantities of each ingredient. The main stages in juice production are as follows :

1. Preparation

This stage involves preparing the raw materials by weighing the ingredients (CMC powder, stabilizer, vitamin C) in the weighing room to create a ready-to-use mixture. According to the RAMY MILK recipe, these ingredients are mixed in a grinder and syrup (Brix degree + water) and fruit concentrate are added.

2. Correction

This stage consists of homogenizing citric acid, flavors, colorants and water in a correction vat containing the initial mixture. Once prepared, a sample is taken to measure physico-chemical parameters (Brix level, acidity, pH) and organoleptic characteristics (taste, odor, color) to ensure juice conformity (**Chen et al., 2013**).

3. Pasteurization

Pasteurization of juice involves using heat to eliminate resistant micro-organisms that represent a risk to public health. Heat is generated externally and transferred to the juice by conduction and convection. By carefully controlling time and temperature (from 80°C to 95°C), pathogens are effectively destroyed and the presence of harmful micro-organisms is considerably reduced (**Chen et al., 2013**).

4. Cooling

The mixture is transferred to buffer tanks to reduce the temperature to 25 °C for two hours in a heat exchanger. This cooling step stops microbial activity (**Chen et al., 2013**).

5. Packaging

When packaging and transporting industrial materials and products, comprehensive protection against climatic, physico-chemical and mechanical factors is crucial. Packaging must remain intact during handling, transport and storage, and resist degradation (**MILLET, 2010**). For

successful packaging, the juice passes through sterile tubes at temperatures between 20°C and 25°C in the packaging room. The cans are also sterilized using peroxide, hot air and drying. The finished product is stored at room temperature (**Graumlich et al., 1986; Vila and Ampuero, 2007**).

III. Bioactive and functional compounds: phenolic compounds and milk proteins

III.1. Phenolic compounds

Phenolic compounds, produced by secondary plant metabolism, are found in various parts of plants (**Lugasi and Hóvári, 2003**). These compounds are found in cell organelles such as vacuoles (e.g. anthocyanins), nuclei, plastids and cell walls (e.g. flavonoids). In addition, phenolic compounds such as lignin are found in cutinized and suberized cell walls (**Reed et al., 2021**). Polyphenols have many functions, including as pigments, flavors and signaling molecules that can attract or repel organisms (**Vermerris and Nicholson, 2007**).

These molecules encompass a vast group of over 8,000 different types, each fulfilling diverse functions such as contributing to cell wall structure, aiding plant growth and development, defending against pathogens and insects, protecting against UV and B rays, and attracting pollinators (**Lugasi and Hóvári, 2003; Vermerris and Nicholson, 2007**).

The hydroxyl groups of phenolic compounds contribute to their acidic nature, although their acidity is relatively low with a pKa generally between 10 and 12. In addition, these compounds are prone to oxidation and are rarely found in their free form in plant tissues (**EL BIR, 2014**).

Phenols are compounds with one or more hydroxyl groups (-OH) directly linked to an aromatic system (**Soto-Hernández et al., 2017**). They are distinguished by their carbon skeleton (number of carbons), their degree of modification (oxidation, hydration, methylation) and their linkages with other molecules such as sugars or other phenolic compounds (**Verdu et al., 2014**).

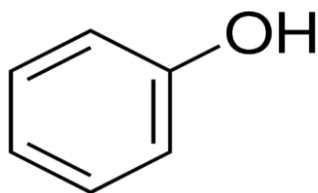


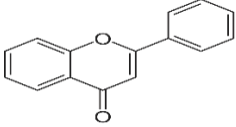
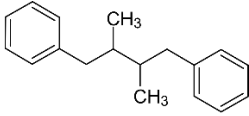
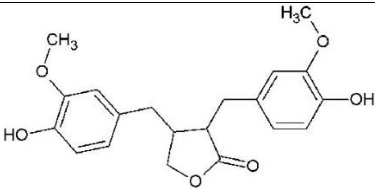
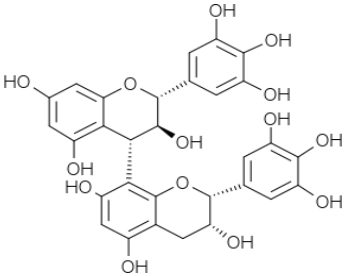
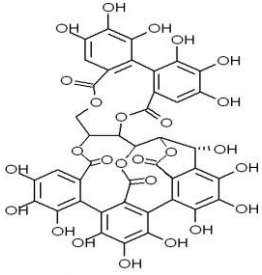
Figure 2: Basic structure of phenol (Soto-Hernández et al., 2017).

III.1.1. Classification of phenolic compounds

Phenolics are divided into flavonoids and non-flavonoids and show great structural diversity. These compounds range from simple C₆ phenols to highly polymerized and altered forms. Particular classes include simple phenols, hydroxybenzoic acids, hydroxycinnamic acids, coumarins, stilbenes, flavonoids, lignans, lignins, condensed tannins and hydrolyzable tannins (Péroumal, 2014; Singla et al., 2019). Examples of phenolic compounds and their structures are shown in **Table I**.

Table I: Different classes of phenolic compounds (Vermerris and Nicholson, 2007; EL BIR, 2014).

Class	Carbon skeleton	Basic structure	Compound (example)
Simple phenols	C ₆		Catechol
Hydroxybenzoic acids	C ₆ -C ₁		<i>p</i> -Hydroxybenzoic acid
Hydroxycinnamic acids	C ₆ -C ₃		Caffeic acid
Stilbenes	C ₆ -C ₂ -C ₆		Resveratrol

Flavonoids	$C_6-C_3-C_6$		Quercetin
Lignans	$(C_6-C_3)_2$		Pinoresinol
Lignins	$(C_6-C_3)_n$		Matairesinol
Condensed tannins	$(C_6-C_3-C_6)_n$		Prodelphinidine
Hydrolysable tannins	$(C_6-C_1)_n$		Castalagine

III.1.2. The health benefits of phenolic compounds

Polyphenols, naturally present in foods or formed during processing, are essential to human health thanks to their antioxidant properties. They scavenge free radicals which reduce the risk of diseases associated with oxidative stress, such as cardiovascular disease and cancer (EL BIR, 2014; Péroumal, 2014).

III.1.3. Flavonoids

With over 4000 known structures (Forkmann and Martens, 2001), flavonoids are the largest group of phenolic compounds present in various sources. These compounds have a 15-carbon skeleton organized in a C₆-C₃-C₆ configuration, comprising two aromatic rings linked by three carbon atoms forming an oxygenated heterocycle (Cutrim and Cortez, 2018).

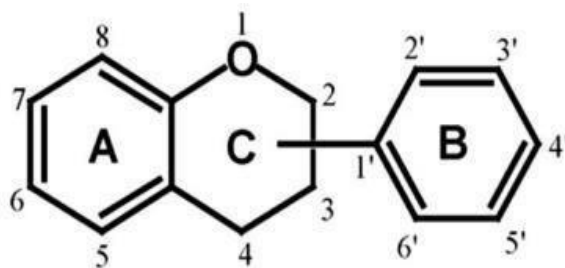
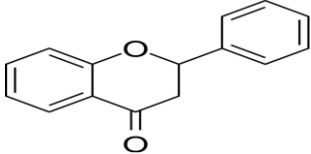
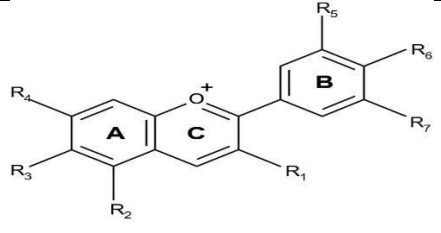
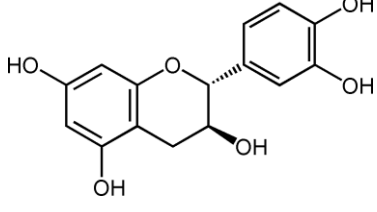
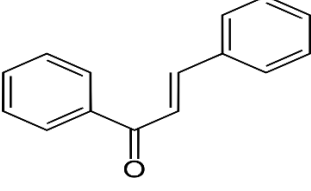


Figure 3: Basic structure of a flavonoid (Cutrim and Cortez, 2018).

The wide range of flavonoids results from variations in the hydroxylation patterns and oxidation state of the central pyran ring, leading to structural diversity. This diversity includes compounds such as flavanols, anthocyanidins, anthocyanins, isoflavones, flavones, flavonols, flavanones and flavanonols (Forkmann and Martens, 2001).

Table II: Different flavonoid classes (Elmouloud, 2016).

Class	Basic structure
Flavones	
Flavonols	
Isoflavones	

Flavanones	
Anthocyanidins	
Catechins	
Chalcones	

III.2. Proteins in dairy products

Dairy products such as fermented milk (L'ben) are mainly characterized by the presence of milk-derived proteins. Caseins and whey proteins make up a significant proportion of the overall protein content of fermented milk.

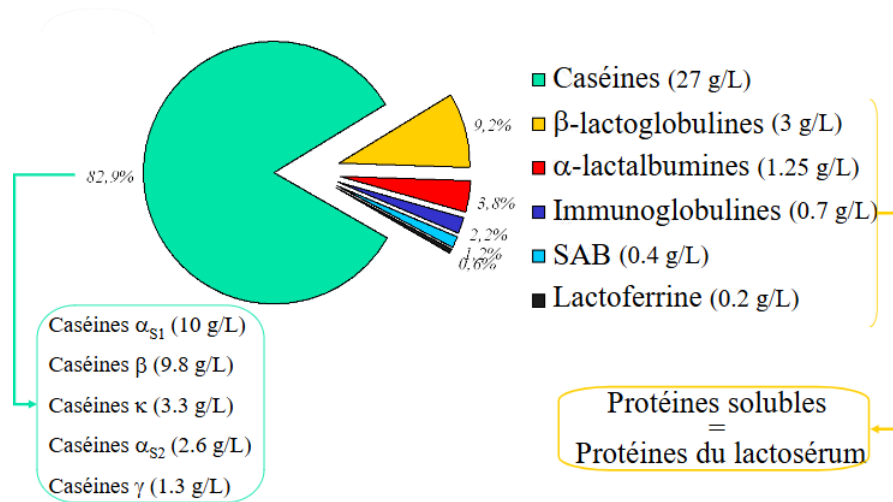


Figure 4: Different protein molecules in dairy products.

III.2.1. Caseins

Caseins are heterogeneous proteins and the predominant class of proteins in the milk of all common dairy species (**Huppertz, 2012**). These proteins precipitate at a temperature of 20°C and a pH of 4.6. There are four main phosphoproteins in this category : α_{S1} -, α_{S2} -, β -, and κ -casein, each with its own amino acid sequence. The combination of casein proteins and calcium phosphate forms large colloidal particles called casein micelles, which improve the mobility of casein molecules and facilitate the dissolution of phosphate and calcium (**Gigli, 2016**).

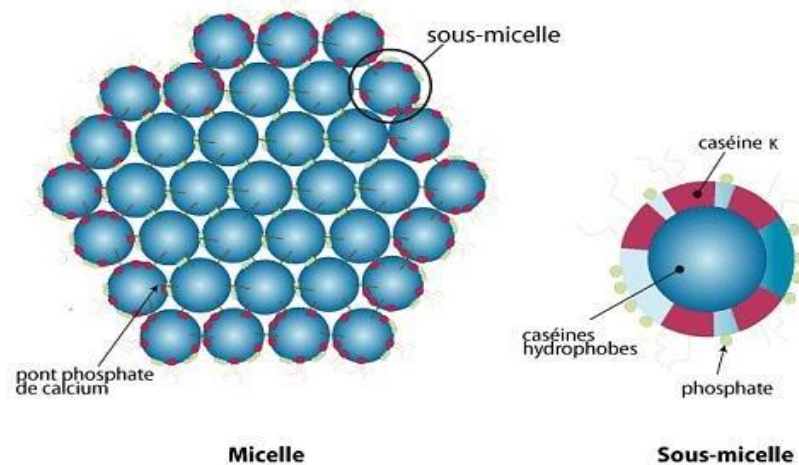


Figure 5: Casein micelle.

III.2.1.1. α -casein

More precisely named α S-casein, this 37 kDa protein has high nutritional properties. It consists of two distinct gene products, α S1- and α S2-casein, which prevent the aggregation and precipitation of various proteins from dairy and non-dairy sources under stress conditions (Treweek, 2012).

III.2.1.2. β -casein

A major component of the casein micelle, this protein has a molecular mass of around 24 kDa and consists of 212 amino acids (Jensen, 2002). It is used as a foaming and emulsifying agent, important in the food industry (O'Connell et al., 2003).

III.2.1.3. κ -casein

Representing around 15% of total casein, this protein plays a crucial role in the formation and stabilization of casein micelles and affects several important technological properties of the milk protein system (Waugh and Von Hippel, 1956).

III.2.2. Whey proteins

Whey proteins are widely used in the food industry due to their functional, biological, bioactive and nutritional characteristics (Jovanović et al., 2005). These proteins are commonly used for enrichment and purification techniques, both in laboratory and industrial settings. Whey proteins are mainly made up of β -lactoglobulin (β -lg) and α -lactalbumin (α -la), which accounts for around 70-80% of total whey protein (Chatterton et al., 2006).

III.2.2.1. α -Lactalbumin

α -Lactalbumin is a small whey protein (14.2 kDa) composed of a single polypeptide chain of 123 amino acids, including 67 essential amino acids. It has two distinct domains: a large α -helical domain and a small β -sheet domain (Jovanović et al., 2005). They are the most stable globular whey fraction protein due to four disulfide bridges in the polypeptide chain and the absence of free -SH groups (Chatterton et al., 2006).

III.2.2.2. β -Lactoglobulin

β -Lactoglobulin is the major whey protein (36.6 kDa) with a defined secondary and tertiary structure, consisting of α -helical, β -sheet and random coil structures (Jovanović et al., 2005). It

binds to several small hydrophobic molecules at a main internal binding site, with additional external sites also identified (**Huppertz, 2012**).

III.2.2.3. Immunoglobulin

Made up of four chains - two heavy chains (50-70 kDa) and two light chains (25 kDa) immunoglobulins play a dual role in the immune system, identifying antigens and initiating mechanisms for their eradication (**Lefranc and Lefranc, 2001**).

III.2.2.4. Serum albumin

Serum albumin is the predominant protein in plasma, with a molecular mass of 65 kDa. It plays a crucial role in many physiological functions, thanks to its diverse properties. Serum albumin is known for its anti-inflammatory capabilities, antioxidant properties, and its role in preventing platelet aggregation, thus helping to maintain homeostasis and prevent various pathologies (**Arques, 2018**).

III.2.2.5. Proteose-peptones

Proteose-peptones represent a specific fraction of whey proteins, obtained after heat treatment at 90°C for 30 minutes, followed by pH adjustment to 4.7. This fraction is divided into two different groups. The peptides in the first group are derived from the degradation of caseins by endogenous milk proteinases, notably plasmin. The second group is made up of other proteins not directly derived from caseins (**Sørensen and Petersen, 1993**).

III.3. Enriching food products with bioactive molecules

Enriching food products with functional ingredients derived from agri-food waste (AFW) is an innovative and sustainable approach to improving the nutritional and functional value of foods. Phenolic compounds, for example, possess a wide range of biological activities, including antibacterial, anti-carcinogenic, anti-inflammatory and antioxidant properties (**Léonil, 2013**).

Similarly, proteins such as caseins and whey proteins play a crucial role in enhancing the nutritional qualities of dairy products. These proteins have anti-hypertensive, anti-thrombotic and antioxidant properties. Integrating these bioactive molecules into food products enables the development of functional foods that contribute to disease prevention (**Léonil, 2013**).

Part II : Material and Methods

II. Material and Methods

II.1. Material and reagents

Our study focused on the recovery of juice and fermented milk (L'ben) waste. Each juice and fermented milk production process generates around 100 L of organic waste after the production tanks have been rinsed with water. The organic waste was collected from the RAMY Milk food industry, located in the El Harrach region of Algiers.

RAMY Milk is a private Algerian company created in 2007 from DELICE FOOD COMPANY (DFC), founded in 2005. DFC operates in the agri-food sector, specializing in the production of soft drinks and dairy products. The production site is located in the Rouïba industrial zone in Algiers, a strategic location for the supply of raw materials and national distribution of finished products.

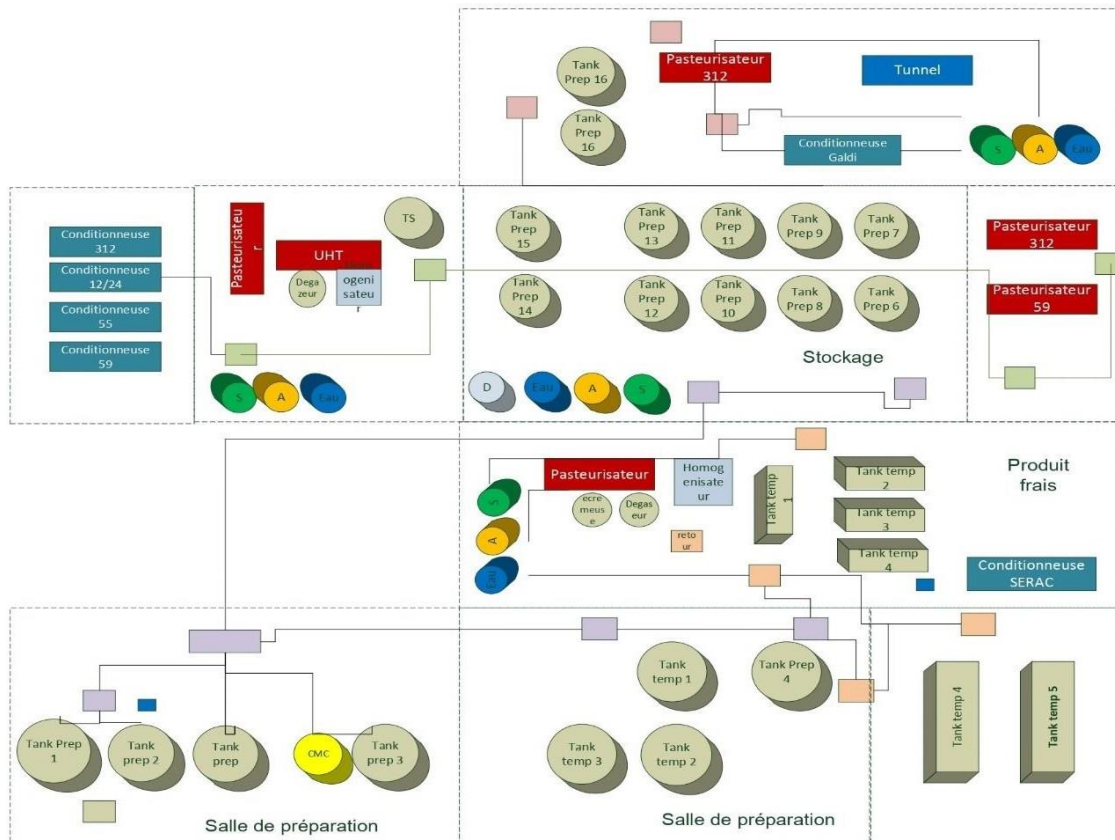


Figure 6: RAMY MILK processing plant layout.

The materials and reagents used in this study are presented in **Tables III** and **IV**.

Table III : Equipment used.

Materials			
Funnels	Decanting ampoules	spectrophotometer cuvettes	Erlenmeyers
Graduated cylinder	Beakers	Watch glass	Test tube rack
Centrifuge	Freeze-dryer	Oven	Rotary evaporator
Analytical balance	Chemical hood	Water bath	Spectrophotometer
Micropipette (100-1000 μ L)	Burette	pH-meter	Vortex
Shaker plate	Wattman paper N ^o 1	Centrifuge tubes	Test tubes

Table IV: Solvents and reagents used.

Reagents	Crude formula	Reagents	Crude formula
Ethanol 96 %	C ₂ H ₆ O	Folin-Ciocalteu	/
n-Hexane	C ₆ H ₁₄	n-Butanol	C ₄ H ₁₀ O
Ethyl acetate	C ₄ H ₈ O ₂	Acetone	C ₃ H ₆ O
Sodium carbonate	Na ₂ CO ₃	Gallic acid	C ₇ H ₆ O ₅
Aluminum chloride	AlCl ₃	Quercetin	C ₁₅ H ₁₀ O ₇
Sulfuric acid	H ₂ SO ₄	Sodium chloride	NaCl
Sodium hydroxide	NaOH	Hydrogen chloride	HCl
Coomassie brilliant blue (G-250)	C ₄₇ H ₄₈ N ₃ NaO ₇ S ₂	Bovine serum albumin (BSA)	/

Phosphoric acid	H_3PO_4	Sodium hydrogen phosphate	Na_2HPO_4
Sodium dihydrogen phosphate	NaH_2PO_4	Potassium ferricyanide	$C_6N_6FeK_3$
Trichloroacetic acid (TCA)	$C_2HCl_3O_2$	Ferric chloride	$FeCl_3$
Ascorbic acid	$C_6H_8O_6$	2,2-diphenyl-1-picrylhydrazyl (DPPH)	$C_{18}H_{12}N_5O_6$
2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS)	$C_{18}H_{18}N_4O_6S_4$	Potassium persulfate	$K_2S_2O_8$

II.2. Methodes

II.2.1. Physico-chemical parameters

II.2.1.1. Titratable acidity

Titratable acidity, also known as total acidity, is an essential method for assessing the concentration of acid present in food products or beverages. This measurement is obtained by titrating the solution with sodium hydroxide (NaOH). Two types of titrations are commonly used :potentiometric titration and colorimetric titration (**Friedrich, 2001**).

The potentiometric acidity titration protocol was developed in accordance with **Friedrich (2001)**. To measure acid content, 10 mL of sample was poured into a 250 mL beaker, to which 100 mL of distilled water was added for dilution. The beaker was then placed on a stirring plate fitted with a magnetic bar to homogenize the solution. A burette containing 25 mL of 0.1 M of NaOH solution is used to slowly titrate the solution, while the pH meter electrode is immersed in the mixture. NaOH is added drop by drop until the pH reaches 8.20, indicating the end of titration.

Once titration is complete, the result is calculated using the following formula:

$$TA (g/100mL) = \frac{V \times N \times \text{meq. wt} \times 100}{1000 \times v}$$

With:

TA: Titratable acidity.

V: Volume of NaOH (mL).

N: NaOH normality (M).

meq.wt : weight of standard in milliequivalents (citric acid : 64, lactic acid: 90).

v: Sample volume.

II.2.1.1. Hydrogen potential (pH)

pH, short for “hydrogen potential”, is a crucial parameter measuring the concentration of free hydrogen ions in a solution (**Friedrich, 2001**).

To determine pH, 50 mL of sample was transferred to a 100 mL beaker. A magnetic bar was inserted to ensure slow, homogeneous stirring of the solution. The pH meter electrode was then submerged in the mixture to measure pH (**Friedrich, 2001**).

II.2.2. Extraction of polyphenolic compounds

Polyphenolic compounds were extracted from juice and juice waste using the liquid-liquid extraction method with solvents of different polarities. Initially, 200 mL of juice or juice waste was introduced into a separating funnel. The process began with the addition of 50 mL n-hexane, which was carefully mixed and left to settle. After formation of the top layer, representing the n-hexane extract, the bottom layer was carefully separated. The lower layer was then combined with 100 mL ethyl acetate, vigorously stirred and the process repeated. After recovery of the lower layer, it was mixed with 100 mL acetone, and the procedure repeated accordingly. Finally, the addition of 100 mL n-butanol concluded the extraction process. The extracts collected were evaporated using a rotary evaporator, then lyophilized and stored at -20°C.

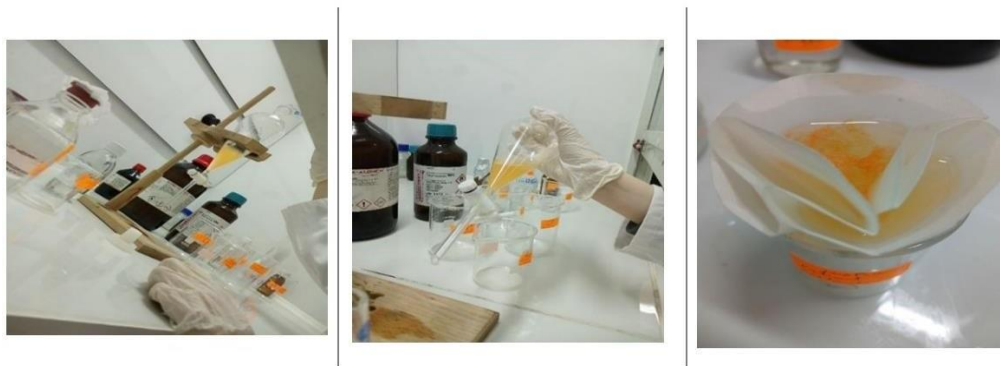


Figure 7: Phenolic compounds extraction steps.

II.2.3. Determination of total polyphenols

Quantification of polyphenols was carried out using the Folin-Ciocalteu method, which relies on the oxidation of phenolic compounds, inducing a change in the color of the sample towards blue (Romulo, 2020).

To quantify phenolic compounds, the protocol described by Singleton and Rossi (1965) was followed. Briefly, 200 μL of sample was mixed with 1 mL of diluted Folin-Ciocalteu solution (1:10 v/v). After a 3-minutes incubation at room temperature, 800 μL of 7.5% (w/v) sodium carbonate solution were added to the mixture and kept in the dark for 30 minutes. Absorbance was measured at 760 nm using a UV-VIS spectrometer.

Polyphenol contents were calculated using the gallic acid calibration curve (Appendix 01), and the amount of polyphenols was expressed in μg EAG/mg of dry extract weight, calculated according to the following formula:

$$C = \frac{C \times V}{m} \times FD$$

With :

C = total polyphenol content (μg EAG /mg extract).

c = EAG extract concentration, obtained from calibration curve.

v = Extract volume (mL).

m = Extract mass (g).

II.2.4. Determination of total flavonoids

The principle of the method is to use aluminum trichloride (AlCl_3) as the reagent. This method relies on the change in color of the solution from clear to yellow, due to the oxidation of flavonoid compounds present in the sample by the AlCl_3 reagent (Dif et al., 2015).

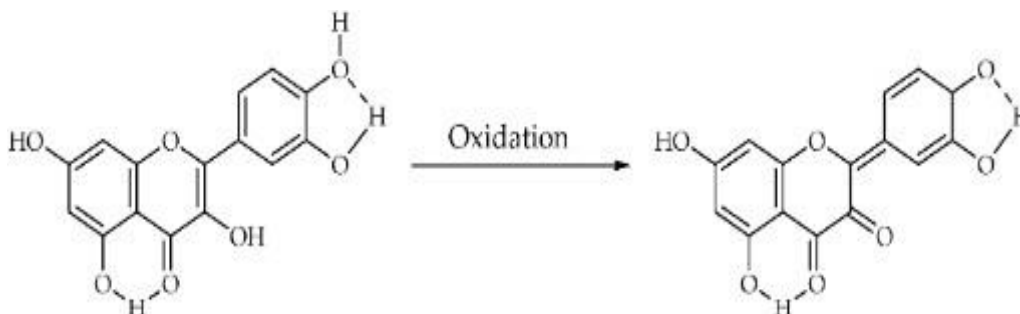


Figure 8: The chemical structure of the quercetin molecule (Gulcin and Alwaseel, 2023).

The flavonoid content of the samples was quantified according to the protocol described by Quettier-Deleu et al. (2000). A 500 μL volume of sample was combined with an equal volume of 2% (w/v) AlCl_3 solution. The mixture was then vortexed and incubated at room temperature for 1 hour in the dark. After incubation, the absorbance of the reaction mixture was measured at 430 nm using a blank. The flavonoid content of the extracts was calculated on the basis of the calibration curve established with quercetin (Appendix 02) and the results were expressed as mg EQ/g dry extract weight.

II.2.5. Protein isolate extraction

The fermented milk protein isolate was extracted by the isoelectric point precipitation method. Specifically, 200 mL of fermented milk waste was poured into a beaker and stirred for 15 minutes to homogenize the solution. The pH was then adjusted to the protein's isoelectric point of 4.6, using a solution of 1 M of HCl. The mixture was transferred to centrifuge tubes and centrifuged at $7000 \times g$ for 10 minutes. After centrifugation, the supernatant was discarded and the pellet containing the proteins was collected and washed several times with distilled water to remove any remaining traces of HCl. The proteins were then lyophilized. Finally, the dry powder obtained was stored at -20°C (Sumner et al., 1981).



Figure 9: Steps in the extraction of fermented milk protein isolate.

II.2.6. Protein content

To measure the protein content of fermented milk protein isolate, the method of **Bradford (1976)** was used. This method is renowned for its speed and sensitivity in protein quantification. It is based on the principle of shifting the wavelength of maximum absorption of the Coomassie Brilliant Blue G-250 dye from 465 nm to 595 nm when it binds to denatured proteins in solution (**Kielkopf et al., 2020**). Under acidic conditions, this dye changes from brown to blue when binding to proteins, forming the basis of the Bradford assay (**He, 2011**).

In this procedure, a volume of 100 μL of appropriately diluted protein isolate solution was combined with 3000 μL of Bradford reagent. The mixture was vortexed and incubated in the dark for 2 minutes. The absorbance of the solution was measured at 595 nm using a UV-VIS spectrophotometer. Bovine serum albumin (BSA) was used as a standard reference. The protein content of the sample was determined from the calibration curve (**Appendix 03**) for BSA and expressed as a percentage.

II.2.7. Functional properties of fermented milk waste protein isolate

II.2.7.1. Emulsifying properties

The emulsifying activity of fermented milk protein isolate was assessed using the turbidimetric method according to the protocol described by **Wang et al. (2018)**. In this procedure, a volume of 3 mL of a 0.2% (w/v) protein isolate solution was combined with 1 mL of sunflower oil. The mixture was vigorously vortexed for 5 minutes to ensure complete mixing of the two phases, followed by centrifugation at $8000 \times g$ for 10 minutes.

Emulsifying capacity was calculated using the following formula:

$$\text{Emulsifying capacity (\%)} = \frac{\text{Emulsion volume}}{\text{Total volume}} \times 100$$

Emulsion stability was measured at intervals of 10, 30, 60 and 90 min after homogenization and calculated using the formula:

$$\text{Emulsion stability(\%)} = \frac{\text{Final emulsion volume}}{\text{Total volume}} \times 100$$

II.2.7.2. Water and oil holding capacities

The water-holding capacity (WHC) and oil-holding capacity (OHC) of fermented milk protein isolate were measured according to the method described by **Stone et al. (2015)**. Specifically, 100 mg of protein isolate was placed in pre-weighed centrifuge tubes, and 1 mL of water or sunflower oil was added. The mixtures were vortexed for 1 minute, then left at room temperature for 30 minutes. After this incubation period, the samples were centrifuged at 8,000 g for 10 minutes. The supernatant was carefully decanted, and the resulting pellet was dried in an oven to remove any trace of water or oil. WHC and OHC were calculated and expressed as grams of water or oil retained per gram of protein sample.

II.2.7.3. Solubility

The solubility of the fermented milk protein isolate was assessed using a modified version of the method described by **Stone et al. (2015)**. In this procedure, 100 mg of protein isolate was mixed with 10 mL of 1M of NaCl solution. The pH of the suspension was adjusted to 7.0 using 0.1 M of NaOH. The mixture was stirred continuously for 1 hour at room temperature, then centrifuged at 7000 × g for 10 minutes. The protein content of the supernatant was measured using the Bradford method, with BSA as standard. Protein solubility (%) was calculated as follows :

$$\text{Solubility (\%)} = \frac{P1}{P0} \times 100$$

With:

P1: protein content of supernatant.

P0: total protein content of the sample.

II.2.8. Antioxidant activity

Assessment of antioxidant activity is crucial for determining the efficacy of phenolic compounds as bioactive substances. Various tests are available for this purpose, including the DPPH and ABTS tests, which are colorimetric methods known for their simplicity, speed of execution. Another commonly used method is the iron reducing power (FRAP) test (Olszowy and Dawidowicz, 2018).

II.2.8.1. The DPPH radical trapping test

The DPPH test is based on the reduction of the DPPH radical, a stable free radical characterized by its intense violet color. When an antioxidant donates an electron or hydrogen atom to the DPPH radical, it is reduced to a non-radical form, resulting in a pale-yellow color (Figure 10). This change in color intensity is measured spectrophotometrically at 517 nm. The decrease in absorbance is directly correlated to the amount of DPPH radical trapped by the antioxidant (Mishra et al., 2012).

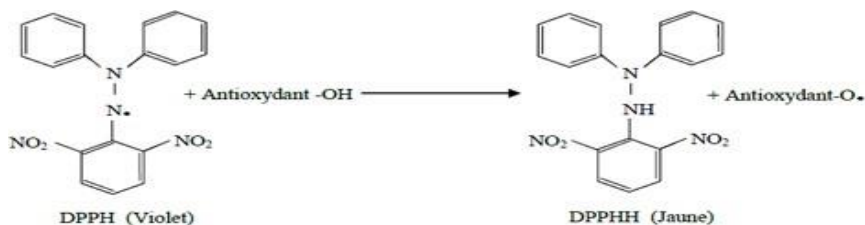


Figure 10: Antioxidant reaction with DPPH (Popovici et al., 2009).

The DPPH radical scavenging capacity of juice and juice waste extracts was assessed using a modified protocol based on the method described by Alara et al. (2018). Briefly, 1 mL of a freshly prepared 0.1 mM DPPH solution was mixed with 100 μ L of samples prepared at different concentrations. The mixtures were incubated in the dark for 30 minutes, after which absorbance was measured at 517 nm using a UV-visible spectrophotometer. Ascorbic acid, a standard antioxidant, served as a positive control and was measured under the same conditions (Appendix 04). The percentage inhibition (I%) of the DPPH radical was calculated using the following formula :

$$I(\%) = \frac{\text{Abs control} - \text{Abs echantiollon}}{\text{Abs control}} \times 100$$

With :

Abs control: absorbance of DPPH solution.

Abs echantillon: absorbance of the sample.

The IC₅₀ value, which indicates the inhibitory concentration required to neutralize 50% of DPPH radicals, serves as an estimate of antioxidant activity. A lower IC₅₀ value means a higher efficacy of the extract. This value is expressed in mg/mL (Boubekri, 2014).

II.2.8.2. ABTS^{•+} radical scavenging test

The ABTS^{•+} radical scavenging activity test is a fast, simple method that can be performed at different pH levels. When ABTS is mixed with an oxidizing agent such as potassium persulfate, it generates a stable ABTS^{•+} radical, which absorbs light at 734 nm. The presence of hydrogen- or electron-donating antioxidants, such as phenolic compounds, in the reaction medium leads to the reduction of the blue-green colored ABTS^{•+} radical to its colorless neutral form (ABTS) (Romulo, 2020).

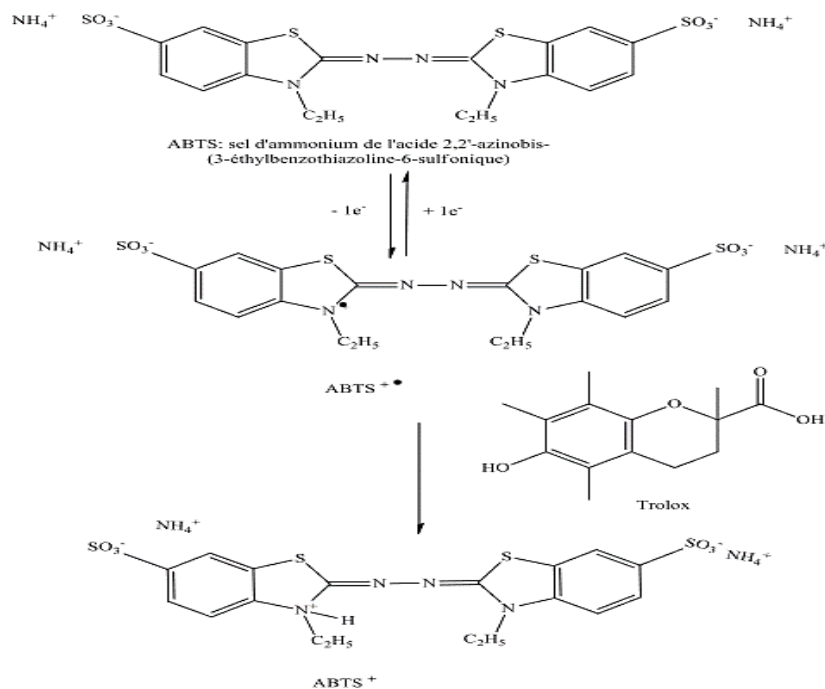


Figure 11: Generation and neutralization of the ABTS^{•+} radical by a hydrogen-donating antioxidant (Marc et al., 2004).

The ABTS^{•+} radical scavenging activity test was carried out as described by **Ilaiyaraja et al. (2015)**, with minor modifications. To generate the ABTS^{•+} radical, a stock solution containing 7 mM ABTS and 2.4 mM potassium persulfate was prepared and left to react in the dark for 16 hours. After radical generation, the stock solution was diluted with ethanol to give an absorbance of 0.7. Next, 1 mL of this freshly diluted ABTS^{•+} solution was added to 100 mL of extracts prepared at different concentrations. The mixtures were incubated in the dark for 10 minutes and absorbance measured at 734 nm. Percent inhibition was calculated as previously described (**II.2.8.1**) and the IC50 value of each extract was determined. Ascorbic acid was used as a positive control (**Appendix 05**).

II.2.8.3. Reducing power of iron (FRAP)

The FRAP method is based on the reduction of ferric ions (Fe³⁺) to ferrous ions (Fe²⁺). When in the presence of reducing agents present in antioxidants, the Fe³⁺/ferricyanide complex is converted to its ferrous form. This reaction produces a cyan-blue color, the intensity of which, measured at 700 nm, reflects the amount of Fe²⁺ formed (**Dudonne et al., 2009**).

The reducing power of the extracts was assessed according to the method described by **Le et al. (2007)**. At different concentrations, 250 µL of sample was mixed with 250 µL of phosphate buffer (0.2 M, pH 6.6) and 250 µL of 1% (w/v) potassium ferricyanide [K₃Fe(CN)₆]. After a 20-minute incubation at 50 °C, 250 µL of 10% trichloroacetic acid (TCA) was added to stop the reaction. The tubes were then centrifuged at 3000 rpm for 10 minutes. Next, 500 µL of each supernatant was mixed with 100 µL of 0.1% (w/v) FeCl₃ solution and left in the dark for 10 minutes before measuring absorbance at 700 nm.

II.2.9. Enrichment of RAMY juice

In our study, RAMY juice was enriched with a phenolic extract and a protein isolate from juice waste and fermented milk waste respectively. The enrichment process involved adding different concentrations of each extract to the juice. The phenolic extract concentrations used were 10 mg/ml, 25 mg/ml and 50 mg/ml, while the protein isolate concentrations were 1.25 mg/ml, 2.5 mg/ml and 5 mg/ml.

Each enriched juice sample was subjected to various tests, including quantification of polyphenolic compounds and proteins, as well as assessment of antioxidant activity using the same tests as mentioned above.

After determining the optimum concentrations of phenolic extract and protein isolate according to their antioxidant activity and the clarity of the juice solution without precipitated proteins (in the case of enrichment with protein isolate), the juice was enriched with both phenolic extract and protein isolate. The final enriched juice was then analyzed for antioxidant activity, protein content and polyphenolic compound content.

III.2.10. Statistical analysis

The experiments described in this study were carried out in triplicate and all data are presented as mean values \pm standard deviation. Statistical analysis was carried out using a one-way ANOVA to compare several groups and determine any significant differences between them. In addition, a two-way ANOVA was performed to assess the influence of two independent variables on the observed results. Significance levels were set at $p < 0.05$.

Part III: Results and discussion

III. Results and discussion

III.1. Physico-chemical parameters of juice, juice waste and fermented milk

III.1.1. pH and titratable acidity

The pH values and titratable acidity of the various samples - juice, juice waste and fermented milk - were measured and calculated, and are shown in **Table V**.

Table V: pH and titratable acidity values of the samples studied.

Parameters	Samples		
	RAMY juice	Juice waste	Fermented milk waste
Titratable acidity	3.13±0.32 ^a	0.051 ± 0.003 ^b	2.75 ± 0.11 ^a
pH	3.27 ± 0.141 ^b	3.79 ± 0.18 ^b	5.40 ± 0.267 ^a

Letters indicate significant differences between samples ($p < 0.05$). Samples with the same letters are not significantly different.

The results showed a significant difference ($p < 0.05$) between the titratable acidity of juice and juice waste, and between juice waste and fermented milk waste. On the other hand, there was a non-significant difference ($p > 0.05$) between the titratable acidity of juice and that of fermented milk waste.

As far as pH levels are concerned, there was no difference between the pH of juice and that of juice waste, while that of fermented milk was higher, at 5.4.

In accordance with the standard established by the RAMY industry, the pH and titratable acidity of juice are both reported at 3.2, which corresponds closely to the results we obtained.

III.2. Extraction yield

In this study, we prepared several juice and juice waste extracts using the liquid-liquid extraction method with solvents of different polarities, including n-hexane, ethyl acetate, acetone and n-butanol. Four extracts were obtained for each sample, and their extraction yields are presented in **Tableau VI**.

Table VI: Extraction yield by liquid-liquid extraction.

Extracts	Yield (%)			
	n-hexane	Ethyl acetate	Acetone	n-butanol
Juice	0.74 ± 0.36 ^c	0.79 ± 0.21 ^c	8.25 ± 0.00 ^b	0.8 ± 0.2 ^c
Juice waste	0.42 ± 0.02 ^c	0.715 ± 0.130 ^c	10.05 ± 0.55 ^a	0.85 ± 0.15 ^c

Letters indicate significant differences between samples ($p < 0.05$). Samples with the same letters are not significantly different.

The results showed that extraction yields were influenced by the solvent used, indicating that the polarity of the solvents used in liquid-liquid extraction had an impact on yield. The highest yield was obtained with acetone as extraction solvent, at $8.25 \pm 0.00\%$ for juice and $10.05 \pm 0.55\%$ for waste juice. For the other extracts using n-hexane, n-butanol and ethyl acetate, there was no significant difference between them for juice and juice waste extracts, and their yields were much lower than those obtained with acetone.

These yield variations underline the importance of solvent polarity in the extraction process. Previous studies by **Muhamad et al. (2014)** have shown that methanol, due to its polarity, offers a better yield. Our results point in the same direction, since acetone, a polar solvent like methanol, also achieves higher yields. This can be attributed to the fact that polyphenols, which are more amphiphilic, dissolve better in polar solvents, explaining the higher yields of acetone extracts compared to those of other solvents.

On the other hand, hexane is a non-polar solvent, making polyphenols less soluble in it. This is in line with the findings of **Muhamad et al. (2014)**. Ethyl acetate, a moderately polar solvent, can dissolve substances of moderate polarity. Although butanol is a highly polar solvent, its extraction yield was lower than that of acetone. This is probably due to the fact that acetone has already extracted most polar substances, while butanol extracts very few.

III.3. Determination of polyphenolic compounds

III.3.1. Total polyphenol content

The polyphenolic content of each extract was determined and the results are presented in **Table VII**.

Table VII : Polyphenolic compounds content of various extracts.

Extracts	Juice ($\mu\text{g GAE/mg extract}$)	Juice waste ($\mu\text{g GAE/mg extract}$)
n-hexane	$0.366 \pm 0.03^{\text{d}}$	$0.392 \pm 0.02^{\text{d}}$
Ethyl acetate	$21.024 \pm 1.20^{\text{a}}$	$10.552 \pm 0.12^{\text{b}}$
Acetone	$2.105 \pm 0.13^{\text{d}}$	$4.692 \pm 0.33^{\text{c}}$
n-butanol	$0.249 \pm 0.14^{\text{d}}$	$19.61 \pm 1.76^{\text{a}}$

Letters indicate significant differences between samples ($p < 0.05$). Samples with the same letters are not significantly different.

The results revealed significant variations in polyphenol content between extracts. Specifically, the ethyl acetate extract of RAMY juice had the highest polyphenolic content, with $21.02 \pm 1.20 \mu\text{g GAE/mg extract}$. Conversely, for juice waste, the n-butanol extract had the highest polyphenolic content with $19.61 \pm 1.76 \mu\text{g GAE/mg extract}$, followed by ethyl acetate extract with $10.55 \mu\text{g GAE/mg extract}$. In contrast, polyphenol content was low in the butanol extract of the juice, as well as in the acetone and n-hexane extracts. Similarly, the polyphenolic content of the acetone and n-hexane extracts of juice waste was also low.

Our study is in line with previous results. **Boussoussa et al. (2014)** reported similar results for the ethyl acetate extract of juice. In addition, **Anokwuru et al. (2011)** found that butanol extracts had the highest polyphenol content in various juice waste samples.

However, it should be noted that **Anokwuru et al. (2011)** observed a higher polyphenol content in acetone extracts than in ethyl acetate extracts, which contrasts with our results. According to **Anokwuru et al. (2011)**, acetone appears to be the optimal solvent for extracting phenolic compounds. Conversely, n-hexane is identified by **Muhamad et al. (2014)** as a solvent with minimal ability to extract polyphenols.

III.3.2. Total flavonoid content

The flavonoid content of each extract is detailed in **Table VIII**. The results show that the n-butanolic extract has the highest flavonoid content, at $4.1 \pm 0.06 \mu\text{g QE/mg extract}$ for waste juice and $0.462 \pm 0.03 \mu\text{g QE/mg extract}$ for juice, in both samples. Thereafter, a slight decrease in flavonoids was observed, with converging levels observed in the ethyl acetate extract of juice waste ($0.52 \pm 0.00 \mu\text{g QE/mg extract}$) and in the hexane extract of juice. Conversely, the lowest flavonoid content was observed in the ethyl acetate extract of juice and n-hexane extract from juice waste.

Table VIII: Flavonoid content of different extracts.

Extracts	Juice ($\mu\text{g QE/mg extract}$)	Juice waste $\mu\text{g QE/mg extract}$
n-hexane	$0.415 \pm 0.02^{\text{bc}}$	$0.102 \pm 0.00^{\text{c}}$
Ethyl acetate	$0.176 \pm 0.02^{\text{c}}$	$0.527 \pm 0.00^{\text{b}}$
Acetone	$0.260 \pm 0.08^{\text{cd}^{\text{c}}}$	$0.359 \pm 0.12^{\text{bd}}$
n-butanol	$0.462 \pm 0.03^{\text{b}}$	$4.10 \pm 0.06^{\text{a}}$

Letters indicate significant differences between samples ($p < 0.05$). Samples with the same letters are not significantly different.

These results are in line with previous studies by **Anokwuru et al (2011)**, who showed that butanol and ethyl acetate gave the highest flavonoid contents, while n-hexane showed poor flavonoid extraction performance.

The preference for butanol and ethyl acetate as primary solvents for flavonoid extraction is consistent with their effectiveness in producing substantial quantities of these bioactive compounds in these investigations.

III.4. Evaluation of antioxidant activities

III.4.1 DPPH radical scavenging activity

The DPPH radical scavenging activity of juice and juice waste extracts was evaluated at a concentration of 10 mg/mL, and the results, presented as inhibition percentages, are shown in **Figure 12**.

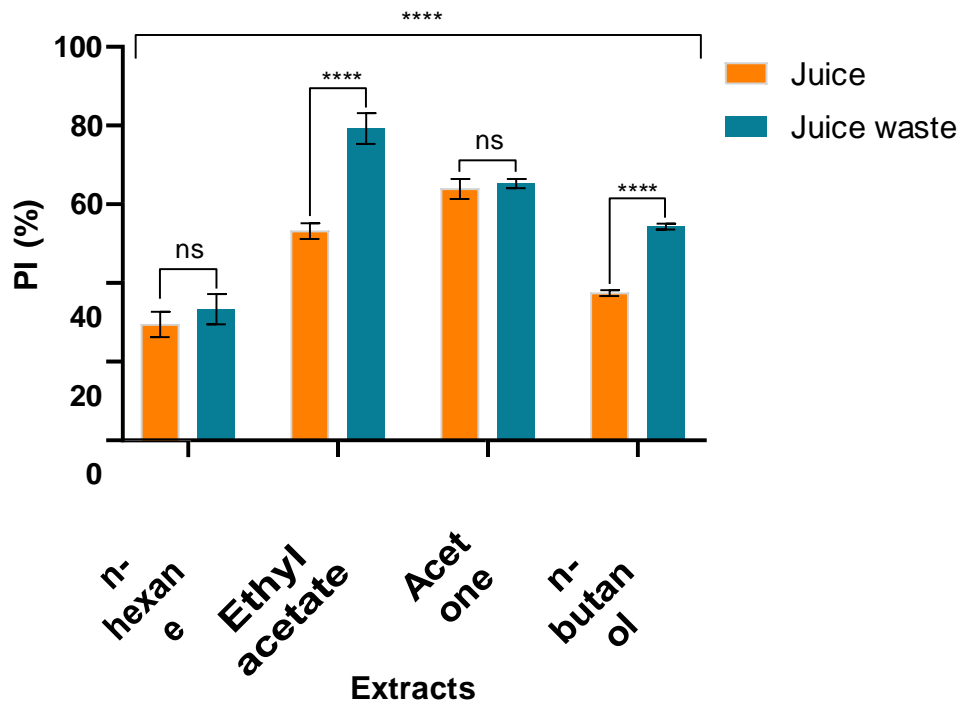


Figure 12: DPPH radical scavenging activity of the different extracts, expressed as percent inhibition (%).

The highest DPPH radical scavenging activity was observed with the ethyl acetate extract of juice waste ($79.27 \pm 3.89\%$), followed by the activity of the acetone extract of juice and juice waste ($63.93 \pm 2.51\%$ and $65.28 \pm 1.134\%$, respectively). The ethyl acetate extract of juice showed slightly lower activity, with a percentage inhibition of $53.22 \pm 1.98\%$. In contrast, juice waste butanol extract showed a higher inhibition percentage of $54.36 \pm 0.77\%$, significantly exceeding that of juice butanol extract ($37.48 \pm 0.79\%$).

Conversely, the n-hexane extract from both samples showed the lowest activity, with inhibition percentages of $29.48 \pm 3.18\%$ and $33.37 \pm 3.81\%$ for juice and juice waste, respectively.

This result shows that the DPPH radical-scavenging activity of the ethyl acetate extract of juice waste is the most potent, although it does not contain the highest amount of phenolic compounds.

III.4.2. ABTS⁺ radical scavenging activity

The ABTS⁺ radical scavenging activity of juice and juice waste extracts was assessed at a concentration of 10 mg/mL, with results expressed as percentage inhibition and presented in **Figure 13**.

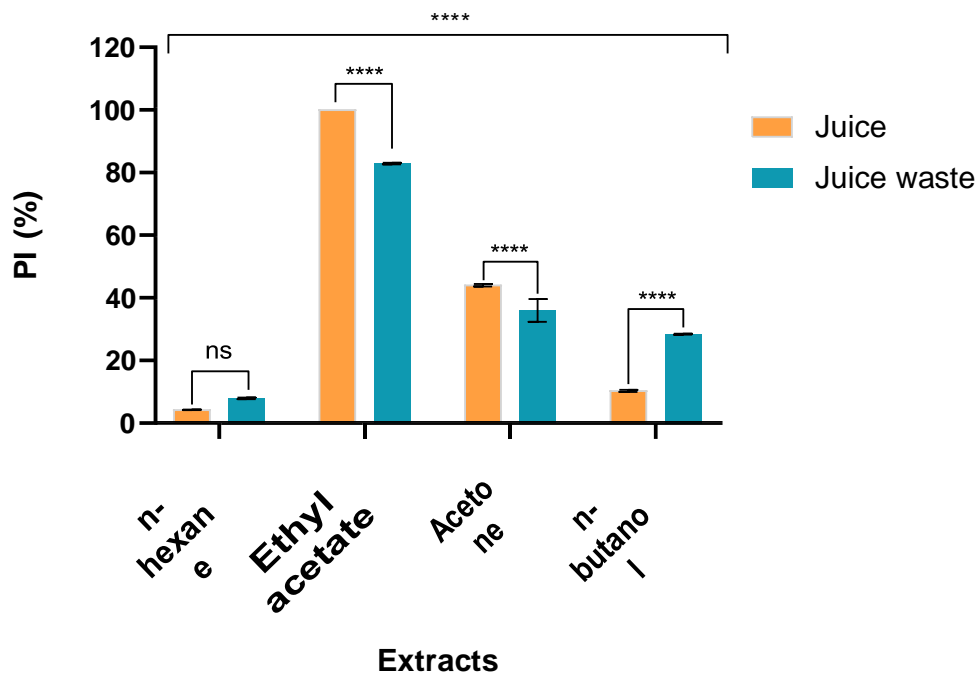


Figure 13: ABTS⁺ radical scavenging activity of the various extracts, expressed as percentage inhibition (%).

For the waste juice extracts, the ethyl acetate extract showed the highest activity with a percentage inhibition of $82.88 \pm 0.24\%$, while the activity of the other extracts was below 40%. The n-hexane extract showed the lowest activity, with a PI of $7.93 \pm 0.24\%$.

Similarly, for juice extracts, ethyl acetate extract showed the highest activity with $100 \pm 0.00\%$, followed by acetone extract with a PI of $44.03 \pm 0.37\%$. The n-hexane extract showed the lowest activity, with a PI of $4.27 \pm 0.022\%$.

III.4.3. Iron reduction capacity (FRAP)

The ability of extracts to reduce iron was determined at a concentration of 10 mg/mL. The activity of the different extracts, measured at 700 nm, is shown in **Figure 14**.

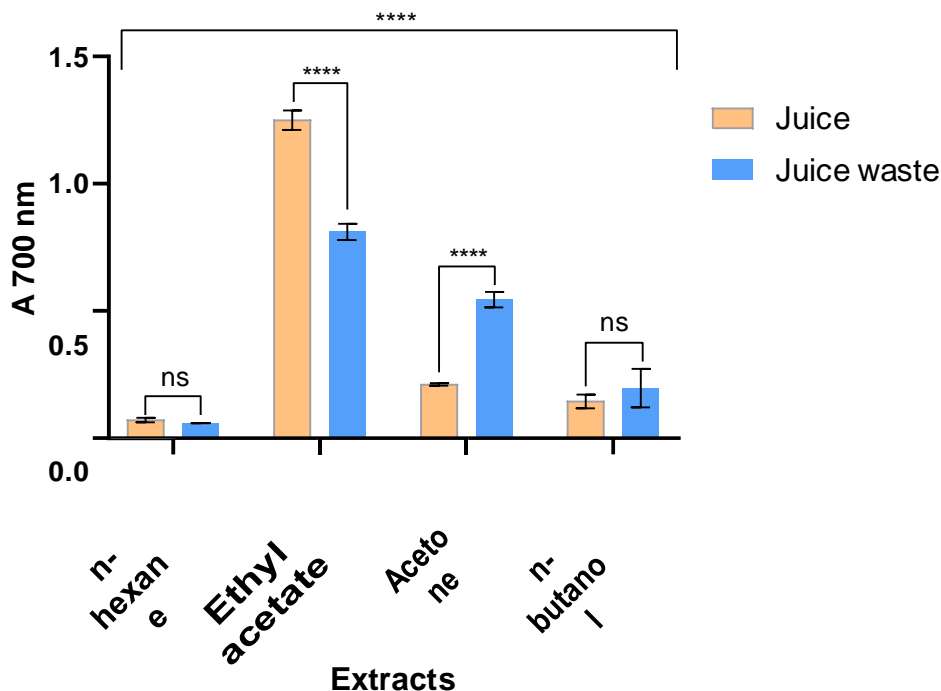


Figure 14: Iron reduction capacity of the various extracts measured at 700 nm.

As with DPPH and ABTS^{•+} radical scavenging activities, the ethyl acetate extract of juice and juice waste showed the highest reducing power, with absorbance values $A_{700\text{nm}} = 1.25 \pm 0.038$ and $A_{700\text{nm}} = 0.811 \pm 0.032$, respectively. The acetone extract of juice waste showed the second highest reducing power with $A_{700\text{nm}} = 0.545 \pm 0.030$, surpassing that of juice acetone extract ($A_{700\text{nm}} = 0.211 \pm 0.005$). In contrast, the reducing activity of n-butanol extracts from both samples was lower, while n-hexane extracts showed the lowest reducing power ($A_{700\text{nm}} = 0.07 \pm 0.008$ for juice and $A_{700\text{nm}} = 0.06 \pm 0.0004$ for juice waste).

III.5. Protein extraction and assay

Protein isolate from fermented milk waste was extracted by a precipitation method, giving an extraction yield of $5 \pm 0.124\%$. The protein content of the isolate was determined using the Bradford method, revealing that it contained $30.74 \pm 2.88\%$ protein, as shown in **Table IX**.

Table IX: Extraction yield and protein content of protein isolate from fermented milk waste.

	Extraction yield (%)	Protein content (%)
Protein isolate	5 ± 0.124	30.74 ± 2.88

III.6. Functional properties of fermented milk waste protein isolate

Proteins offer a variety of functional properties, including solubility, emulsifying capacities and the ability to retain water and oil. These attributes significantly influence the behavior of proteins in food systems (Benzitoune et al., 2022).

III.6.1. Solubility and water/oil retention

The solubility of fermented milk protein isolate, as well as its water and oil retention capacities, are detailed in **Table X**. The results indicate that the solubility of the protein isolate is notably low, at $6.772 \pm 1.02\%$, which is significantly lower than the results of **Wong and Kitts (2003)** for buttermilk solids ($33.66 \pm 7.7\%$), non-fat dry milk ($34.6 \pm 3.5\%$), soy protein isolate ($14.3 \pm 2.8\%$), dried egg white ($18.0 \pm 1.3\%$) and dried egg yolk ($20.9 \pm 6.7\%$). This low solubility can be attributed to the precipitation conditions, as the proteins were precipitated at their isoelectric point, where they tend to self-aggregate and form clusters. Even when tested at pH 7, solubility remained low. Studies have shown that proteins are generally more soluble under strongly acidic or basic conditions.

Table X: Solubility and water/oil holding capacity of fermented milk waste protein isolate.

	Solubility (%)	Water holding capacity (g/g)	Oil holding capacity (g/g)
Protein isolate	6.772 ± 1.02	3.1 ± 0.5	4.5 ± 0.1

The ability of proteins to absorb water and oil is crucial to their function as food additives, improving the overall quality of protein-containing foods. These properties not only improve food quality, but also have an impact on shelf life, texture and flavor.

Fermented milk protein isolate exhibited a water retention capacity of 3.1 ± 0.5 g/g, which is higher than that of buttermilk solids (0.85 ± 0.13 g/g), non-fat dehydrated milk (0.55 ± 0.01 g/g)

and dehydrated egg yolk (0.96 ± 0.07 g/g), but lower than that of soy protein isolate (4.60 ± 0.14 g/g), as reported by **Wong and Kitts (2003)**.

The oil retention capacity of the protein isolate was 4.5 ± 0.1 g/g, which is higher than the capacities reported for non-fat dehydrated milk (3.03 ± 0.05 g/g) and buttermilk solids (1.17 ± 0.04 g/g) by **Wong and Kitts (2003)**.

III.6.2. Emulsifying activity and emulsion stability

The ability of proteins to form an emulsion by simultaneously interacting with oil and water is known as emulsifying activity. This process is mainly influenced by factors such as ionic charge and hydrophobicity of the protein surface (**Zhang et al., 2020**). The emulsifying activity of protein isolate from fermented milk waste is $31.25 \pm 1.25\%$, which is lower than that of buttermilk solids (50%), as reported by **Wong and Kitts (2003)**.

Emulsion stability, over a given period, refers to the protein's ability to resist disruptive changes in emulsions, such as creaming, flocculation and coalescence. Emulsion stability remains constant throughout the test period (30 min, 60 min, 90 min) with a value of $25 \pm 0.05\%$ (**Table XI**). This indicates that the proteins are relatively effective in preventing oil droplet aggregation.

Table XI: Emulsifying activity and emulsion stability of fermented milk waste protein isolate.

	Emulsifying activity (%)	Emulsion stability (%)		
		30 min	60 min	90 min
Protein isolate	31.25 ± 1.25	25 ± 0.05	25 ± 0.05	25 ± 0.05

The consistent emulsifying stability of isolated proteins over time confirms the presence of hydrophobic interactions that facilitate protein-protein interactions, leading to the formation of robust interfacial films as deduced by **Wong and Kitts (2003)**.

III.7. Enrichment of RAMY juice with ethyl acetate extract of juice waste

Four extracts were prepared from juice waste: n-hexane, ethyl acetate, acetone and n-butanol. Polyphenol and flavonoid contents and antioxidant activities were determined for each extract. Ethyl acetate extract was selected for enrichment of RAMY fruit juice because of its higher

antioxidant activity than other extracts. Juice enrichment with this extract was tested at variable concentrations of 10 mg/mL, 25 mg/mL and 50 mg/mL.

III.7.1. Determination of polyphenolic compounds

The total TPC and TFC contents of unenriched and enriched juices, with different concentrations of ethyl acetate extract, were determined and are presented in **Table XII**.

The results show that the polyphenol and flavonoid contents of the enriched juice increase significantly with increasing ethyl acetate extract concentrations. At a concentration of 50 mg/mL, the enriched juice had a polyphenol content of $60.027 \pm 9.04 \mu\text{g GAE/mg extract}$ and a flavonoid content of $13.03 \pm 1.15 \mu\text{g QE/mg extract}$. These values are significantly higher than those of unenriched juice, whose polyphenol and flavonoid contents are $0.309 \pm 0.102 \mu\text{g GAE/mg extract}$ and $0.038 \pm 0.00 \mu\text{g QE/mg extract}$ respectively. These results show that enrichment of RAMY fruit juice with ethyl acetate extract increased its polyphenol and flavonoid content.

Table XII: Polyphenol and flavonoid contents of enriched and unenriched juices with different concentrations of ethyl acetate extract.

Concentration of ethyl acetate extract	Total polyphenol content ($\mu\text{g GAE/mg extract}$)	Total flavonoids content ($\mu\text{g QE/mg extract}$)
10 mg/mL	10.498 ± 0.08^b	1.147 ± 0.07^b
25 mg/mL	15.956 ± 0.38^b	2.930 ± 0.07^b
50 mg/mL	60.027 ± 9.04^a	13.039 ± 1.15^a
Unenriched juice	0.309 ± 0.102^c	0.038 ± 0.00^c

Letters indicate significant differences between samples ($p < 0.05$). Samples with the same letters are not significantly different.

III.7.2. Antioxidant activity

The activity of enriched juice was assessed using the DPPH and ABTS^{•+} radical scavenging tests, as well as the FRAP test.

The DPPH radical scavenging activity of juice enriched with three different concentrations of ethyl acetate extracts was assessed at different dilutions of enriched juice. Inhibition percentages and IC₅₀ values for each enriched juice are shown in **Figure 15**.

The results show a significant increase in DPPH radical inhibition with increasing concentration. Surprisingly, juice enriched with 10 mg/mL ethyl acetate extract showed the highest DPPH radical scavenging activity, followed by the 25 mg/mL concentration, while the 50 mg/mL concentration showed the lowest activity. IC₅₀ values confirm these results, with the 10 mg/mL concentration showing the lowest value (3.31 ± 0.239 mg/mL), followed by the 25 mg/mL concentration (9.98 ± 0.442 mg/mL), and the highest value for the 50 mg/mL concentration (19.20 ± 0.982 mg/mL).

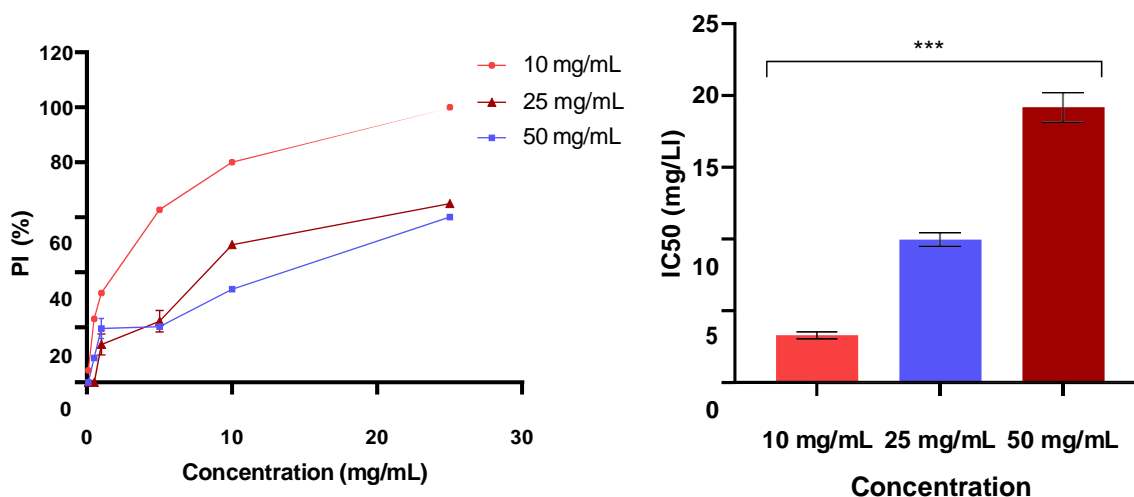


Figure 15: DPPH radical scavenging activity and IC₅₀ values of samples at different concentrations (**p < 0.001).

Similar results were observed for ABTS^{•+} radical scavenging activity, with the 10 mg/mL concentration showing the highest activity (IC₅₀ = 2.241 ± 0.139 mg/mL), followed by 25 mg/mL (IC₅₀ = 3.68 ± 0.257 mg/mL) and the lowest activity for the 50 mg/mL concentration (IC₅₀ = 6.62 ± 0.345 mg/mL) (**Figure 16**).

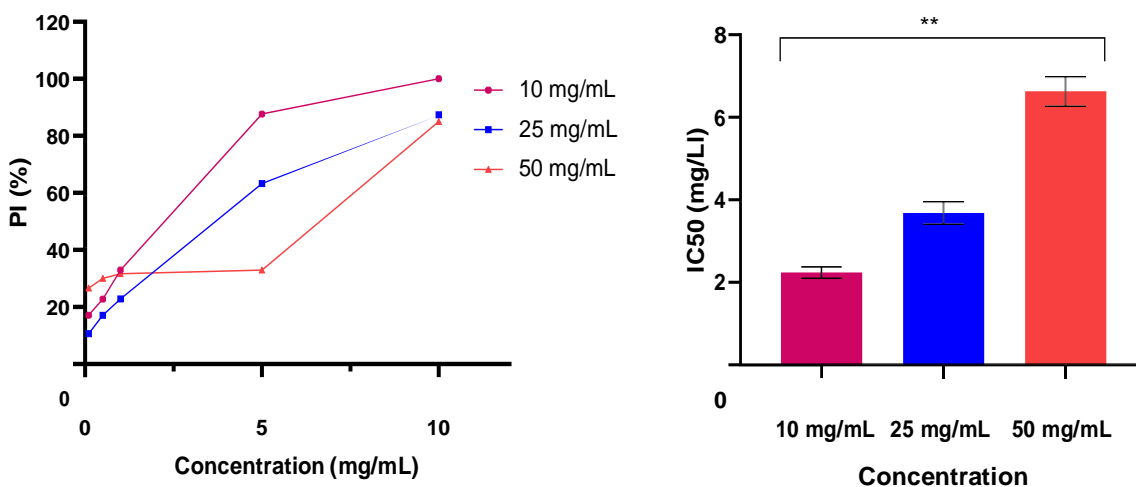


Figure 16: ABTS⁺ radical scavenging activity and IC₅₀ values of samples at different concentrations (**p < 0.01).

The reducing power (FRAP) of juice enriched with ethyl acetate extract followed similar trends. The 10 mg/mL concentration showed the highest reducing power, with the 25 mg/mL concentration showing comparable activity. However, the 50 mg/mL concentration showed significantly lower activity (Figure 17).

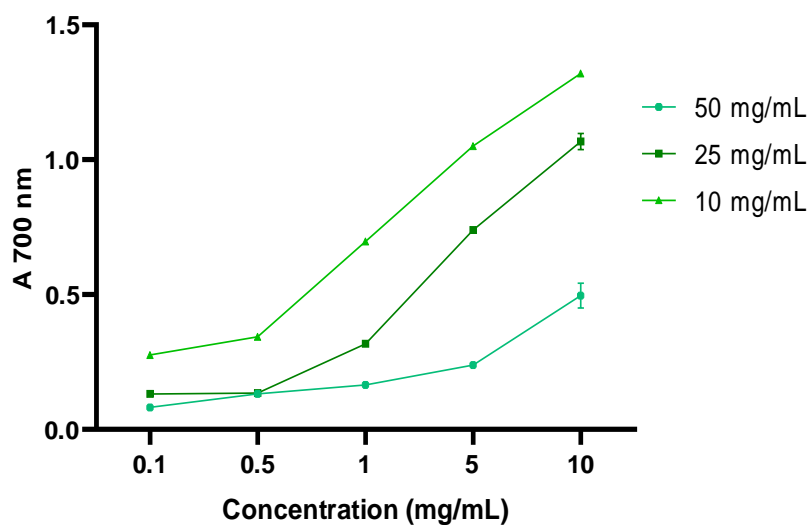


Figure 17: Reducing power of samples at different concentrations.

These results suggest that increasing the concentration of ethyl acetate from 10 mg/mL to

50 mg/mL reduces the antioxidant activity of the juice. This indicates an inverse relationship,

where higher concentrations of ethyl acetate may cause juice polyphenols to act more as pro-oxidants than antioxidants.

III.8. Enrichment of RAMY juice with fermented milk waste protein isolate

RAMY fruit juice was enriched with different concentrations of fermented milk waste protein isolate (1.25 mg/mL, 2.5 mg/mL, 5 mg/mL and 10 mg/mL). The protein content of enriched and non-enriched juices was then quantified, the results being presented in **Table XII**.

Table XIII: Protein content of unenriched juice and juice enriched with different concentrations of fermented milk waste protein isolate.

Concentration of protein isolate from fermented milk waste	Protein content (%)
1.25	4.585 ± 0.36 ^c
2.5	14.958 ± 2.81 ^b
5	15.562 ± 1.26 ^b
10	30.048 ± 3.59 ^a
Unenriched juice	0.427 ± 0.006 ^d

Letters indicate significant differences between samples ($p < 0.05$). Samples with the same letters are not significantly different.

Unenriched juice had a protein content of $0.427 \pm 0.006\%$. Enriching the juice with increasing concentrations of fermented milk waste protein isolate significantly increased the protein content, reaching $30.048 \pm 3.59\%$ at a concentration of 10 mg/mL.

It should be noted that while higher concentrations increase the protein content of the juice, protein precipitation occurs at concentrations of 10 mg/mL, 5 mg/mL and 2.5 mg/mL. This precipitation is due to the acid pH of the juice, which is close to the isoelectric point of the protein isolate. However, enrichment of the juice with 1.25 mg/mL did not cause protein precipitation, resulting in fully soluble proteins.

III.9. Enrichment of RAMY juices with fermented milk waste protein isolate and juice waste ethyl acetate extract

Ethyl acetate extract of juice waste, at a concentration of 10 mg/mL, was chosen to enrich RAMY juice because of its higher antioxidant activity than the other concentrations tested. In addition, fermented milk waste protein isolate was chosen at a concentration of 1.25 mg/mL because of its greater solubility in juice. These two extracts were combined to enrich RAMY fruit juice.

III.9.1. Total polyphenol, flavonoid and protein contents of enriched juice

Quantification of polyphenols, flavonoids and proteins in juice enriched with ethyl acetate extract of juice waste and protein isolate of fermented milk waste revealed significant increases in these compounds compared with unenriched juice. Enriched juice reached values of 39.12 ± 1.71 for protein content, 43.030 ± 2.02 μg GAE/mg extract for total polyphenols and 13 ± 0.47 μg QE/mg extract for flavonoids (**Table XIII**).

Notably, these values are much higher than those obtained for juice enriched with ethyl acetate extract alone or protein isolate alone tested at the same concentrations. This suggests that the combination of ethyl acetate extract and protein isolate is an effective approach for significantly increasing the levels of these metabolites in juice.

Table XIV: Protein, total polyphenol and flavonoid content of juices unenriched and enriched with protein isolate and ethyl acetate extract.

	Enriched juice	Unenriched juice
Protein content (%)	39.12 ± 1.71 ****	0.427 ± 0.006
Total polyphenol content (μg GAE/mg extract)	43.030 ± 2.02 ****	0.309 ± 0.102
Total flavonoid content (μg QE/mg extract)	13 ± 0.47 ****	0.038 ± 0.00

**** $p < 0.0001$ compared with control group (Unenriched juice)

III.9.2. Antioxidant activity

The antioxidant activity of juice enriched with ethyl acetate extract (10 mg/mL) and protein isolate (1.25 mg/mL) was tested and compared with that of unenriched juice. In terms of ABTS⁺

radical scavenging activity, enriched juice showed notable activity, with an IC₅₀ value of 6.13 ± 0.237 mg/mL, while non-enriched juice had a comparable IC₅₀ value of 7.13 mg/mL.

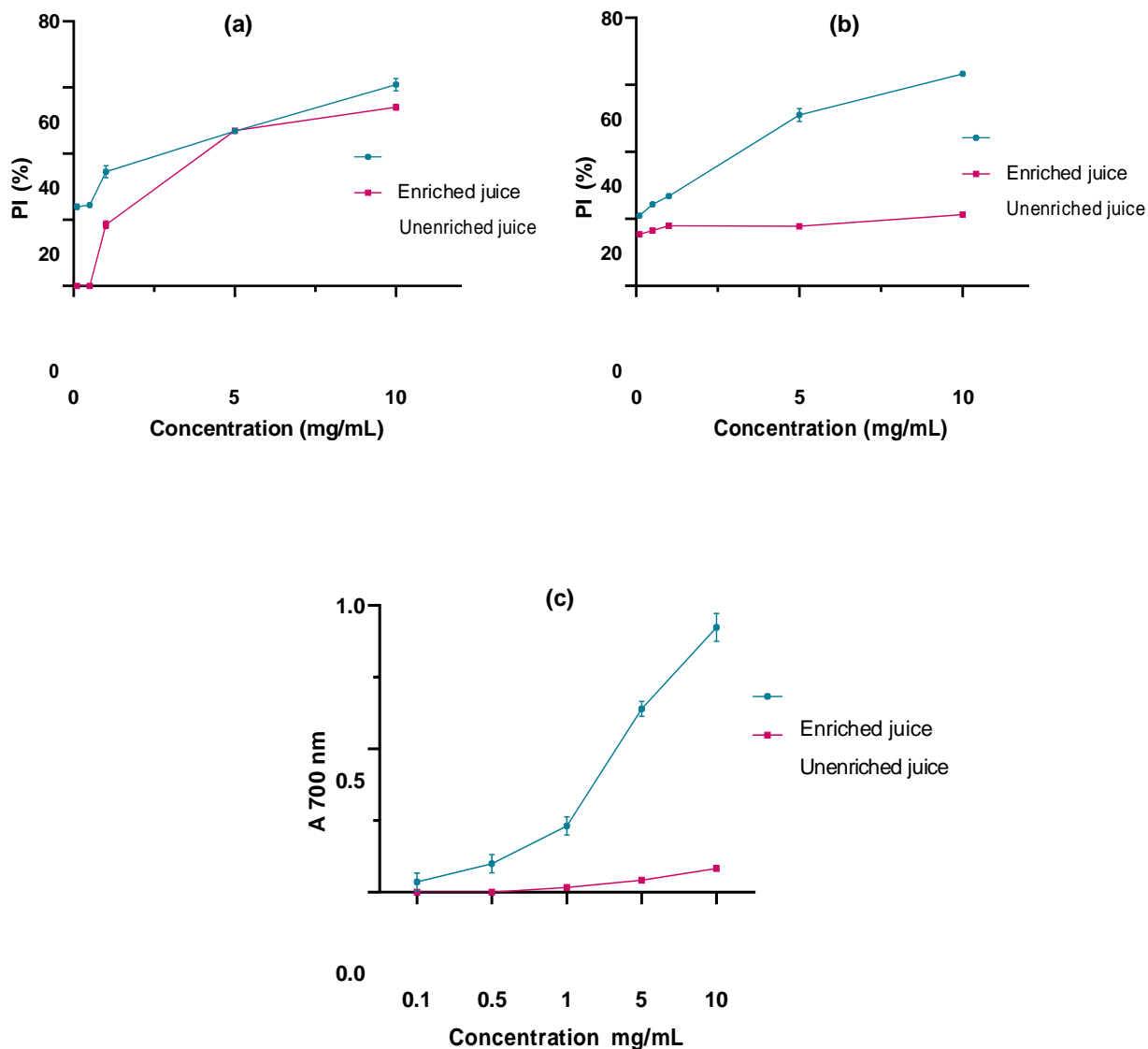


Figure 18: ABTS⁺ radical scavenging activity (a), DPPH radical scavenging activity (b) and reducing power (c) of the samples.

Regarding DPPH radical scavenging activity, the enriched juice also showed good activity, with an IC₅₀ value of 4.83 ± 0.317 mg/mL. In contrast, the non-enriched juice showed such low activity that it failed to reach 50% inhibition, preventing calculation of the IC₅₀. When these IC₅₀ values were compared with those of ascorbic acid, a synthetic antioxidant, ascorbic acid showed much stronger activity, with IC₅₀ values of 0.051 ± 0.003 mg/mL for the ABTS assay and 0.039

± 0.024 mg/mL for the DPPH assay.

In the FRAP test, enriched juice showed significantly greater reducing activity than unenriched juice. At a concentration of 10 mg/mL, enriched juice had an absorbance at 700 nm of 0.924 ± 0.04 , compared with 0.083 ± 0.001 for unenriched juice.

Table XV: IC₅₀ values for enriched and non-enriched juice and ascorbic acid.

	IC ₅₀ (mg/mL)	
	ABTS ^{•+} test	DPPH test
Unenriched juice	7.13 ± 0.038^a	/
Enriched Juice	6.13 ± 0.237^a	4.82 ± 0.317^a
Ascorbic acid	0.051 ± 0.003^b	0.039 ± 0.024^b

Letters indicate significant differences between samples ($p < 0.05$). Samples with the same letters are not significantly different.

When comparing the antioxidant activity of juice enriched with both protein isolate and ethyl acetate extract to that of juice enriched only with ethyl acetate extract, the latter showed better antioxidant activity. This suggests that the combination of protein isolate and ethyl acetate extract reduced the overall antioxidant efficacy of the juice.

General conclusion and perspectives

General conclusion and perspectives

Organic waste from juice production and the RAMY dairy industry is an abundant source of valuable bioactive molecules for the food industry. This research focused on the valorization of these wastes in order to improve the nutritional value and bioactive substance content of RAMY juices.

Our study focused on the extraction of phenolic compounds and proteins from two waste samples, obtaining a satisfactory quantity of these bioactive molecules. We analyzed the protein content for its functional properties, including emulsifying activity, solubility and water and oil retention capacity. In addition, we assessed phenolic compounds, total polyphenols and flavonoids, as well as antioxidant activity through three tests: DPPH and ABTS^{•+} radical scavenging activity, and ferric reducing power.

The results indicate that the highest total phenolic content was found in the ethyl acetate extract (21.024 ± 1.20 μg GAE/mg extract) for the RAMY juice and in the butanolic extract (19.613 ± 1.76 μg GAE/mg extract) for the waste juice. The butanolic extract also showed the highest total flavonoid content (4.1 ± 0.06 μg QE/mg extract), compared with juice waste (0.46 ± 0.03 μg QE/mg extract) and RAMY juice. The protein content of the juice was $30.74 \pm 2.88\%$.

The selection of the best extract for enrichment of RAMY juice was based on the high antioxidant activity observed in the ethyl acetate extract at a concentration of 10 mg/mL and on its higher protein content, which showed better solubility at a concentration of 1.25 mg/mL. After enrichment, phenolic and flavonoid contents increased significantly to 43.30 ± 2.02 μg GAE/mg and 13 ± 0.47 μg QE/mg respectively, compared with the unenriched juice, which had values of 0.309 ± 0.10 μg GAE/mg and 0.38 ± 0.00 μg QE/mg. Similarly, protein content improved considerably, reaching $39.12 \pm 1.71\%$ after enrichment, compared with $0.427 \pm 0.006\%$ in unenriched juice.

Enriched RAMY juice demonstrated good antioxidant activity across all three tests. The IC₅₀ values were 4.83 ± 0.317 mg/mL for DPPH radical scavenging, 6.13 ± 0.237 mg/mL for ABTS^{•+} radical scavenging, and $A_{700\text{ nm}} = 0.924 \pm 0.04$ for FRAP. In comparison, the unenriched

juice had IC₅₀ values of 7.13 ± 0.038 mg/mL for ABTS⁺ radical scavenging, an absorbance of 0.083 ± 0.001 at 700 nm in the FRAP assay, and no activity against DPPH radicals.

The enrichment of RAMY juice with these bioactive molecules resulted in a product rich in antioxidants, potentially offering antihypertensive and antithrombotic benefits and protecting against diseases such as cardiovascular disease and cancer.

To further optimize and validate our results, future research should focus on the following:

- Explore the possibility of extending the extraction and enrichment process to industrial applications to ensure commercial viability and consistent product quality.
- Carry out detailed sensory analyses, including taste, flavor, texture and odor evaluations, to ensure that fortified juice meets consumer expectations.
- Carry out longitudinal studies to evaluate the health benefits of fortified RAMY juice, examining its potential to prevent or mitigate chronic diseases such as cardiovascular disease, cancer and metabolic disorders.
- Develop research to identify and extract other valuable bioactive compounds present in waste streams. These additional compounds could have further health benefits and enhance the functional value of fortified juice.

Bibliographic references

References

- Acourene, S., Ammouche, A., Djaafri, K., 2008. Valorisation des rebuts de dattes par la production de la levure boulangère, de l'alcool et du vinaigre. *Sciences & Technologie. C, Biotechnologies*, 38-45.
- Alara, O.R., Abdurahman, N.H., Ukaegbu, C.I., Azhari, N.H., 2018. Vernonia cinerea leaves as the source of phenolic compounds, antioxidants, and anti-diabetic activity using microwave-assisted extraction technique. *Industrial Crops and Products* 122, 533-544.
- Alimentarius, C.d.C., 2022. Norme générale pour les jus et les nectars de fruits, 247-2005.
- Aliouche, S., Kehila, Y., Benkahoul, L., 2017. Modalités de sélection des sites d'enfouissement technique en Algérie et leur prise en charge par les instruments d'aménagement du territoire et d'urbanisme. *Environnement, Ingénierie & Développement*.
- Anokwuru, C., Anyasor, G., Ajibaye, O., Fakoya, O., Okebugwu, P., 2011. Effect of extraction solvents on phenolic, flavonoid and antioxidant activities of three nigerian medicinal plants. *Nature and Science* 9, 53-61.
- Arques, S., 2018. Albumine sérique et maladies cardiovasculaires: une revue approfondie de la littérature, *Annales de Cardiologie et d'Angéiologie*. Elsevier, pp. 82-90.
- Baiano, A., 2014. Recovery of biomolecules from food wastes—A review. *Molecules* 19, 14821-14842.
- Benzitoune, N., Kadri, N., Adouane, M., Berkani, F., Abbou, A., Dahmoune, F., Remini, H., Bensmail, S., 2022. Pine nuts (*Pinus pinea* L.) as a potential novel plant-based source of functional protein isolates: Optimization of alkali extraction conditions, evaluation of functional properties, and biochemical characterization. *Journal of Food Processing and Preservation* 46, e16471.
- Berger, J., 2003. Enrichissement des aliments en micronutriments: élément d'une stratégie intégrée de lutte contre les carences en micronutriments, en particulier en fer, dans les pays en développement. *Voies alimentaires d'amélioration des situations nutritionnelles, Ouagadougou*, 564-576.
- Boubekri, C., 2014. Etude de l'activité antioxydante des polyphénols extraits de *Solanum melongena* par des techniques électrochimiques. Université Mohamed Khider Biskra.

- Boussoussa, H., Hamia, C., Djeridane, A., Boudjeniba, M., Yousfi, M., 2014. Effect of different solvent polarity on extraction of phenolic compounds from Algerian *Rhanterium adpressum* flowers and their antimicrobial and antioxidant activities. *Current Chemical Biology* 8, 43-50.
- Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry* 72, 248-254.
- Chatterton, D.E., Smithers, G., Roupas, P., Brodkorb, A., 2006. Bioactivity of β -lactoglobulin and α -lactalbumin—Technological implications for processing. *International Dairy Journal* 16, 1229-1240.
- Chen, Y., Yu, L.J., Rupasinghe, H.V., 2013. Effect of thermal and non-thermal pasteurisation on the microbial inactivation and phenolic degradation in fruit juice: A mini-review. *Journal of the Science of Food and Agriculture* 93, 981-986.
- Cutrim, C.S., Cortez, M.A.S., 2018. A review on polyphenols: Classification, beneficial effects and their application in dairy products. *International Journal of Dairy Technology* 71, 564-578.
- Damien, A., 2004. *Guide du traitement des déchets*. Dunod Paris.
- de Reynal, B., 2009. *Les additifs alimentaires. Additifs et auxiliaires de fabrication dans les industries agroalimentaires (4e ed.)*, 3.
- Desqueyroux, H., André, J.-C., 2018. Recyclage et réutilisation. *Environnement, Risques & Santé* 17, 189-190.
- Díaz-García, M., Castellar, M.R., Obón, J.M., 2013. Juices and non-alcoholic beverages, *Comprehensive Analytical Chemistry*. Elsevier, pp. 439-459.
- Dif, M., Benchiha, H., Mehdadi, Z., Benali-Toumi, F., Benyahia, M., Bouterfas, K., 2015. Quantification study of polyphenols in different organs of *Papaver rhoeas* L. *Phytothérapie* 13, 314-319.
- Dudonne, S., Vitrac, X., Coutiere, P., Woillez, M., Mérillon, J.-M., 2009. Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. *Journal of agricultural and food chemistry* 57, 1768-1774.

- EL BIR, M., 2014. contribution à la valorisation des produits et sous produits de l'olivier (*olea europaea* L): application chimiométrique à la détermination de l'origine géographique des huiles d'olives et valorisation des composés phénoliques des noyaux d'olives marocaines.
- Elmouloud, B., 2016. Extraction des polyphénols et étude des activités antioxydante et antibactérienne de quelques plantes Sahariennes. Thèse présentée en vue de l'obtention d'un diplôme de doctorat en science
- Forkmann, G., Martens, S., 2001. Metabolic engineering and applications of flavonoids. *Current opinion in biotechnology* 12, 155-160.
- Friedrich, J.E., 2001. Titratable activity of acid tastants. *Current protocols in food analytical chemistry*, G2. 1.1-G2. 1.7.
- Gigli, I., 2016. Milk proteins: From structure to biological properties and health aspects. BoD—Books on Demand.
- Graumlich, T.R., Marcy, J.E., Adams, J., 1986. Aseptically packaged orange juice and concentrate: a review of the influence of processing and packaging conditions on quality. *Journal of Agricultural and Food Chemistry* 34, 402-405.
- Gulcin, İ., Alwasel, S.H., 2023. DPPH radical scavenging assay. *Processes* 11, 2248.
- He, F., 2011. Bradford protein assay. *Bio-protocol*, e45-e45.
- Hou, C., 2016. Le droit des déchets: étude comparatiste France-Chine. Université Paris-Saclay (ComUE).
- Huppertz, T., 2012. Chemistry of the caseins, *Advanced Dairy Chemistry: Volume 1A: Proteins: Basic Aspects*, 4th Edition. Springer, pp. 135-160.
- Ilaiyaraja, N., Likhith, K., Babu, G.S., Khanum, F., 2015. Optimisation of extraction of bioactive compounds from *Feronia limonia* (wood apple) fruit using response surface methodology (RSM). *Food chemistry* 173, 348-354.
- Jensen, R.G., 2002. The composition of bovine milk lipids: January 1995 to December 2000. *Journal of dairy science* 85, 295-350.
- Jovanović, S., Barać, M., Maćej, O., 2005. Whey proteins-properties and possibility of application. *Mljekarstvo* 55, 215-233.
- Kielkopf, C.L., Bauer, W., Urbatsch, I.L., 2020. Bradford assay for determining protein concentration. *Cold Spring Harbor Protocols* 2020, pdb. prot102269.

- Krasnova, T., 2018. Water treatment in food industry. *Tekhnika i Tekhnologiya Pishchevykh Proizvodstv* 48.
- Le, K., Chiu, F., Ng, K., 2007. Identification and quantification of antioxidants in *Fructus lycii*. *Food chemistry* 105, 353-363.
- Lefranc, M.-P., Lefranc, G., 2001. *The immunoglobulin factsbook*. Academic press.
- Léonil, J., 2013. Peptides bioactifs du lait: bénéfice-santé. *Cholé Doc* 135, np.
- Liu, Z., de Souza, T.S., Holland, B., Dunshea, F., Barrow, C., Suleria, H.A., 2023. Valorization of food waste to produce value-added products based on its bioactive compounds. *Processes* 11, 840.
- Lugasi, A., Hóvári, J., 2003. Antioxidant properties of commercial alcoholic and nonalcoholic beverages. *Food/Nahrung* 47, 79-86.
- Mahongnao, S., Sharma, P., Nanda, S., 2023. Conversion of waste materials into different by-products of economic value, *Waste Management and Resource Recycling in the Developing World*. Elsevier, pp. 665-699.
- Marc, F., Davin, A., Deglene-Benbrahim, L., Ferrand, C., Baccaunaud, M., Fritsch, P., 2004. Méthodes d'évaluation du potentiel antioxydant dans les aliments. *médecine/sciences* 20, 458-463.
- Melini, V., Melini, F., Luziatelli, F., Ruzzi, M., 2020. Functional ingredients from agri-food waste: Effect of inclusion thereof on phenolic compound content and bioaccessibility in bakery products. *Antioxidants* 9, 1216.
- MILLET, P., 2010. *Liquides alimentaires-Organiser un atelier de conditionnement*.
- Mishra, K., Ojha, H., Chaudhury, N.K., 2012. Estimation of antiradical properties of antioxidants using DPPH assay: A critical review and results. *Food chemistry* 130, 1036-1043.
- Mondal, M.I.H., Yeasmin, M.S., Rahman, M.S., 2015. Preparation of food grade carboxymethyl cellulose from corn husk agrowaste. *International Journal of Biological Macromolecules* 79, 144-150.
- Muhamad, N., Muhmed, S.A., Yusoff, M.M., Gimnun, J., 2014. Influence of solvent polarity and conditions on extraction of antioxidant, flavonoids and phenolic content from *Averrhoa bilimbi*. *Journal of Food Science and Engineering* 4, 255-260.

- Naik, B., Kumar, V., Rizwanuddin, S., Chauhan, M., Gupta, A.K., Rustagi, S., Kumar, V., Gupta, S., 2023. Agro-industrial waste: a cost-effective and eco-friendly substrate to produce amylase. *Food Production, Processing and Nutrition* 5, 30.
- Nath, P.C., Ojha, A., Debnath, S., Sharma, M., Nayak, P.K., Sridhar, K., Inbaraj, B.S., 2023. Valorization of food waste as animal feed: a step towards sustainable food waste management and circular bioeconomy. *Animals* 13, 1366.
- Nzihou, A., 2010. Toward the valorization of waste and biomass. *Waste and Biomass Valorization* 1, 3-7.
- O'Connell, J., Grinberg, V.Y., De Kruif, C., 2003. Association behavior of β -casein. *Journal of Colloid and Interface Science* 258, 33-39.
- Olszowy, M., Dawidowicz, A.L., 2018. Is it possible to use the DPPH and ABTS methods for reliable estimation of antioxidant power of colored compounds? *Chemical Papers* 72, 393-400.
- Péroumal, A., 2014. Caractérisation des fruits et de la pulpe de six accessions de *Mammea americana*: Aptitude à la transformation des fruits et caractérisation des composés phénoliques de la pulpe. *Antilles-Guyane*.
- Popovici, C., Saykova, I., Tylkowski, B., 2009. Evaluation de l'activité antioxydant des composés phénoliques par la réactivité avec le radical libre DPPH. *Revue de génie industriel* 4.
- Quettier-Deleu, C., Gressier, B., Vasseur, J., Dine, T., Brunet, C., Luyckx, M., Cazin, M., Cazin, J.-C., Bailleul, F., Trotin, F., 2000. Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. *Journal of ethnopharmacology* 72, 35-42.
- Reed, J.D., de Freitas, V., Quideau, S., Andersen, O., Barron, D., Bidel, L., Cheynier, V., Chèze, C., Comte, G., Daayf, F., 2021. *Recent Advances in Polyphenol Research*. Wiley Online Library.
- René, M., 2009. *Le traitement des Dechets*. Lavoisier.
- Romulo, A., 2020. The principle of some in vitro antioxidant activity methods, *IOP Conference Series: Earth and Environmental Science*. IOP Publishing, p. 012177.
- Sadh, P.K., Duhan, S., Duhan, J.S., 2018. Agro-industrial wastes and their utilization using solid state fermentation: a review. *Bioresources and Bioprocessing* 5, 1-15.

- Singla, R.K., Dubey, A.K., Garg, A., Sharma, R.K., Fiorino, M., Ameen, S.M., Haddad, M.A., Al-Hiary, M., 2019. Natural polyphenols: Chemical classification, definition of classes, subcategories, and structures. Oxford University Press, pp. 1397-1400.
- Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture* 16, 144-158.
- Socas-Rodríguez, B., Álvarez-Rivera, G., Valdés, A., Ibáñez, E., Cifuentes, A., 2021. Food by-products and food wastes: Are they safe enough for their valorization? *Trends in Food Science & Technology* 114, 133-147.
- Sørensen, E.S., Petersen, T.E., 1993. Purification and characterization of three proteins isolated from the proteose peptone fraction of bovine milk. *Journal of Dairy Research* 60, 189-197.
- Soto-Hernández, M., Tenango, M.P., García-Mateos, R., 2017. Phenolic compounds: natural sources, importance and applications. *BoD—Books on Demand*.
- Squillaci, G., La Cara, F., Roseiro, L.B., Marques, I.P., Morana, A., 2021. Agro-industrial wastes as bioactive molecules source. *Chemical Engineering Transactions* 86, 37-42.
- Stone, A.K., Karalash, A., Tyler, R.T., Warkentin, T.D., Nickerson, M.T., 2015. Functional attributes of pea protein isolates prepared using different extraction methods and cultivars. *Food research international* 76, 31-38.
- Sumner, A., Nielsen, M., Youngs, C., 1981. Production and evaluation of pea protein isolate. *Journal of Food Science* 46, 364-366.
- Treweek, T.M., 2012. Alpha-casein as a molecular chaperone. *Milk protein*, 85-119.
- Valencia-Hernandez, L.J., Wong-Paz, J.E., Ascacio-Valdés, J.A., Chávez-González, M.L., Contreras-Esquível, J.C., Aguilar, C.N., 2021. Procyanidins: From agro-industrial waste to food as bioactive molecules. *Foods* 10, 3152.
- Verdu, C.F., Guyot, S., Childebrand, N., Bahut, M., Celton, J.-M., Gaillard, S., Lasserre-Zuber, P., Troggio, M., Guilet, D., Laurens, F., 2014. QTL analysis and candidate gene mapping for the polyphenol content in cider apple. *PLoS One* 9, e107103.
- Vermerris, W., Nicholson, R., 2007. Phenolic compound biochemistry. Springer Science & Business Media.
- Vila, N., Ampuero, O., 2007. The role of packaging in positioning an orange juice. *Journal of Food Products Marketing* 13, 21-48.

- Vilas-Boas, A.A., Pintado, M., Oliveira, A.L., 2021. Natural bioactive compounds from food waste: Toxicity and safety concerns. *Foods* 10, 1564.
- Wang, L., Zhang, B., Xiao, J., Huang, Q., Li, C., Fu, X., 2018. Physicochemical, functional, and biological properties of water-soluble polysaccharides from *Rosa roxburghii* Tratt fruit. *Food Chemistry* 249, 127-135.
- Waugh, D.F., Von Hippel, P.H., 1956. κ -Casein and the stabilization of casein micelles. *Journal of the American Chemical Society* 78, 4576-4582.
- Wong, P., Kitts, D., 2003. A comparison of the buttermilk solids functional properties to nonfat dried milk, soy protein isolate, dried egg white, and egg yolk powders. *Journal of dairy science* 86, 746-754.
- Zhang, Z., He, S., Liu, H., Sun, X., Ye, Y., Cao, X., Wu, Z., Sun, H., 2020. Effect of pH regulation on the components and functional properties of proteins isolated from cold-pressed rapeseed meal through alkaline extraction and acid precipitation. *Food Chemistry*, 126998.

Appendix

Appendix 01: Gallic acid calibration curve.

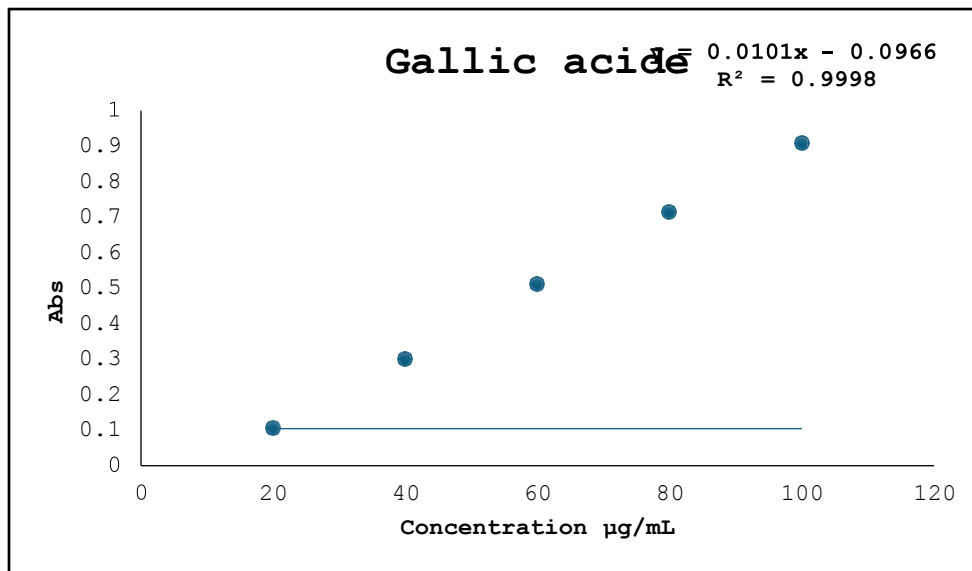


Figure 19: Gallic acid calibration curve used to determine polyphenols.

Appendix 02: Quercetin calibration curve.

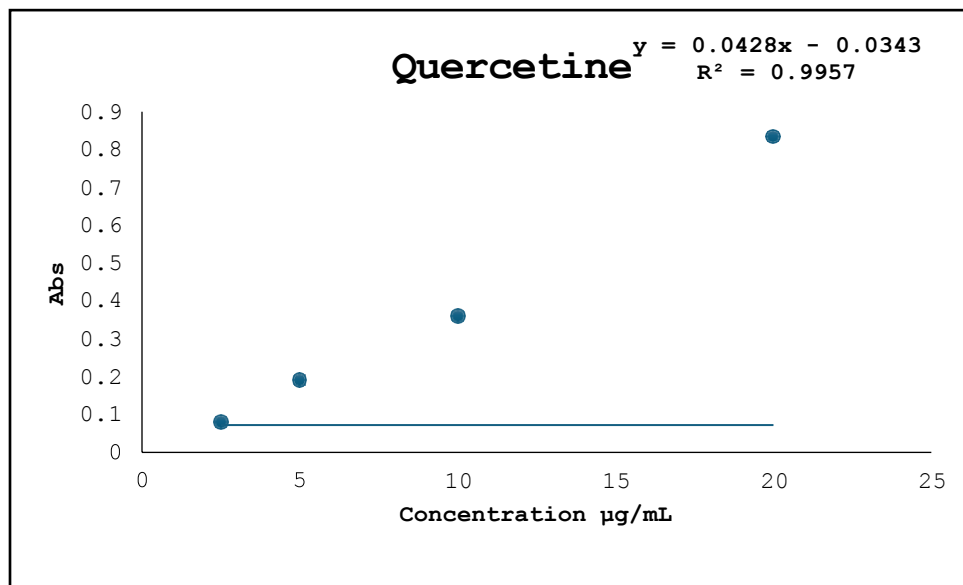


Figure 20: Quercetin calibration curve used to determine total flavonoid content.

Appendix 03: BSA calibration curve.

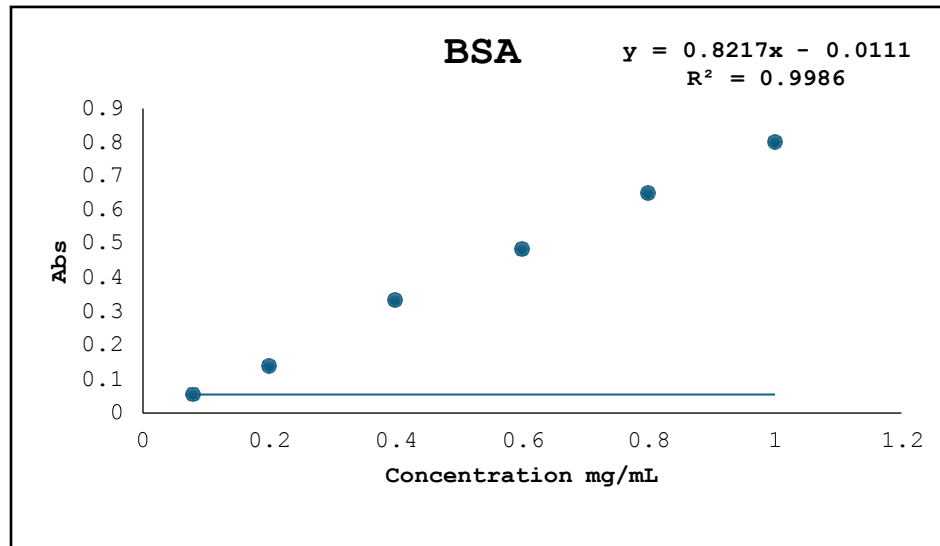


Figure 21: BSA calibration curve used to determine total protein content.

Appendix 04: DPPH radical scavenging activity of ascorbic acid.

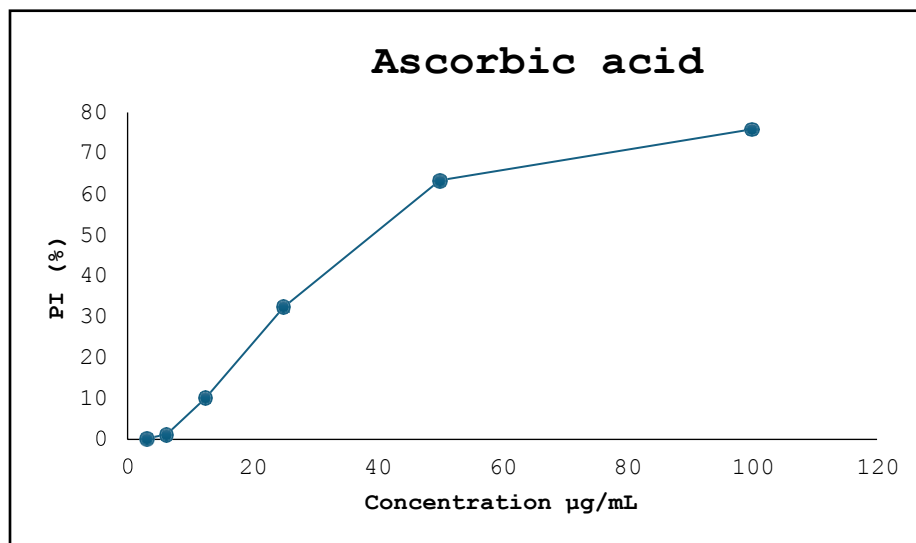


Figure 22: DPPH radical scavenging activity of ascorbic acid

Appendix 05: ABTS^{•+} radical scavenging activity of ascorbic acid.

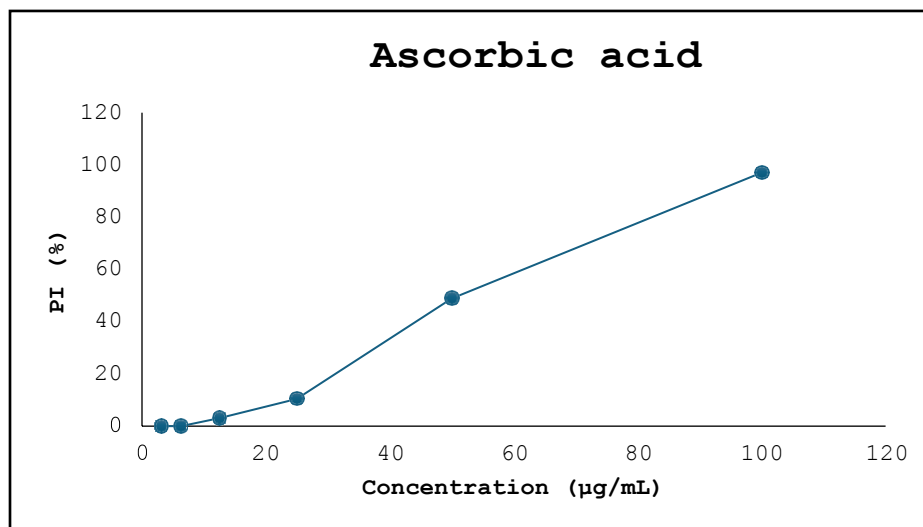


Figure 22: ABTS⁺ radical scavenging activity of ascorbic acid.

Abstract

The agri-food industry generates organic waste rich in bioactive compounds, which poses environmental problems. This study aimed to improve the nutritional value of RAMY juice by enriching it with phenolic compounds and proteins extracted from juice and fermented milk waste. Phenolic and protein contents were analyzed and their antioxidant activities assessed using ABTS, FRAP and DPPH tests. The results showed that the ethyl acetate extract of the RAMY juice had the highest phenolic content ($21.024 \pm 1.20 \mu\text{g GAE/mg}$), while the butanolic extract of the juice waste had the highest flavonoid content ($4.1 \pm 0.06 \mu\text{g QE/mg}$). Enriched juice showed a significant increase in phenolic compounds ($43.30 \pm 2.02 \mu\text{g GAE/mg}$) and flavonoids ($13 \pm 0.47 \mu\text{g QE/mg}$), with a protein content of $39.12 \pm 1.71\%$. The enriched juice demonstrated substantial antioxidant activity, with IC50 values of $4.83 \pm 0.317 \text{ mg/mL}$ for DPPH, $6.13 \pm 0.237 \text{ mg/mL}$ for ABTS⁺, and an absorbance of 0.924 ± 0.04 at 700 nm in the FRAP assay. This study highlights the potential for using organic waste to improve the nutritional profile and bioactive compound content of food products.

Keywords: Organic waste, RAMY juice, phenolic compounds, proteins, Enrichment, Antioxidant activity.

Résumé

L'industrie agroalimentaire génère des déchets organiques riches en composés bioactifs, ce qui pose des problèmes environnementaux. Cette étude vise à améliorer la valeur nutritionnelle du jus de RAMY en l'enrichissant avec des composés phénoliques et des protéines extraits du jus et des déchets de lait fermenté. Les teneurs en composés phénoliques et en protéines ont été analysées et leurs activités antioxydantes évaluées à l'aide des tests ABTS, FRAP et DPPH. Les résultats ont montré que l'extrait d'acétate d'éthyle du jus RAMY avait la teneur phénolique la plus élevée ($21,024 \pm 1,20 \mu\text{g EAG/mg}$), tandis que l'extrait butanolique des déchets de jus avait la teneur en flavonoïdes la plus élevée ($4,1 \pm 0,06 \mu\text{g EQ/mg}$). Le jus enrichi a montré une augmentation significative des composés phénoliques ($43,30 \pm 2,02 \mu\text{g EAG/mg}$) et des flavonoïdes ($13 \pm 0,47 \mu\text{g EQ/mg}$), avec une teneur en protéines de $39,12 \pm 1,71\%$. Le jus enrichi a démontré une activité antioxydante substantielle, avec des valeurs IC50 de $4,83 \pm 0,317 \text{ mg/mL}$ pour DPPH, $6,13 \pm 0,237 \text{ mg/mL}$ pour ABTS⁺, et une absorbance de $0,924 \pm 0,04$ à 700 nm dans le test FRAP. Cette étude met en évidence le potentiel d'utilisation des déchets organiques pour améliorer le profil nutritionnel et la teneur en composés bioactifs des produits alimentaires.

Mots-clés : Déchets organiques, jus de RAMY, composés phénoliques, protéines, enrichissement, activité antioxydante.

ملخص

تولد صناعة الأغذية الزراعية نفايات عضوية غنية بالمركبات النشطة بيولوجيًا، مما يسبب مشاكل بيئية. هدفت هذه الدراسة إلى تحسين القيمة الغذائية لعصير رامي من خلال إثرائه بالمركبات الفينولية والبروتينات المستخلصة من العصير ومخلفات الحليب المخمر. تم تحليل محتويات الفينول والبروتين وتقييم أنشطتها المضادة للأكسدة باستخدام اختبارات ABTS و FRAP و DPPH. وأظهرت النتائج أن مستخلص أسيتات الأثيل من عصير رامي كان يحتوي على أعلى محتوى فينولي ($21.024 \pm 1.20 \mu\text{g EAG/mg}$)، بينما كان مستخلص البوتانولي من فضلات العصير يحتوي على أعلى محتوى فالفونويد ($4.1 \pm 0.06 \mu\text{g EQ/mg}$) وأظهر العصير المخضب زيادة كبيرة في المركبات الفينولية ($43.30 \pm 2.02 \mu\text{g EAG/mg}$) والالفونويدات ($13 \pm 0.47 \mu\text{g EQ/mg}$) مع محتوى بروتيني بنسبة 39.12 ± 1.71%. وأظهر العصير المخضب نشاطا كيميائيا مضادا للأكسدة، حيث بلغت قيم IC50 $4.83 \pm 0.317 \text{ ملغم/مل}$ لـ DPPH، و $6.13 \pm 0.237 \text{ ملغم/مل}$ لـ ABTS⁺، وامتصاصية 0.924 ± 0.04 عند 700 نانومتر في اختبار FRAP. تسلط هذه الدراسة الضوء على إمكانية استخدام النفايات العضوية لتحسين المظهر الغذائي والمحتوى المركب النشط بيولوجيًا للمنتجات الغذائية.

الكلمات المفتاحية: النفايات العضوية، عصير رامي، المركبات الفينولية، البروتينات، التخفيف، النشاط المضاد للأكسدة.