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Trace Elements as Contaminants and Nutrients

Edited by
Agostinho Almeida, Edgar Pinto and Cristina Couto

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Preface to ”Trace Elements as Contaminants and Nutrients”

Scientific data about trace elements has advanced remarkably in recent years, both in terms of their metabolism and functions. Acting mainly as cofactors of enzymatic systems, several trace elements play a crucial role in several physiological processes in the human organism, from cell metabolism to the immune response and gene expression. In contrast, it is also well recognized that excessive exposure to trace elements can be detrimental and even fatal.

The benefits of trace elements, but also their impact as potential toxicants, in the food sector was the topic chosen for this reprint, entitled “Trace Elements as Contaminants and Nutrients“, addressing precisely those two main topics: trace elements as contaminants and trace elements as nutrients.

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Editorial

Trace Elements as Contaminants and Nutrients

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Knowledge about trace elements has evolved remarkably in recent decades, both in terms of their metabolism and their functions. Acting mainly as cofactors of enzymatic systems, several trace elements play an essential role in numerous physiological processes in the human organism, from cell metabolism to the immune response and gene expression, among others. On the other hand, it is also well known that excessive exposure to trace elements can be highly harmful and even fatal.

The benefits of trace elements but also their impact as potential toxicants in the food sector was the topic chosen for this Special Issue of the *Foods* journal, entitled "Trace Elements as Contaminants and Nutrients". It comprises seven research papers [1–7] and one review article [8], addressing precisely those two main topics: trace elements as contaminants and trace elements as nutrients.

Konieczynski et al. [1] investigated the potential role of trace elements in the claimed antidiabetic effect of some medicinal plants. For this purpose, they compared the trace element content of infusions prepared from medicinal plants traditionally used in the treatment of diabetes and a control group (medicinal plants without this therapeutic indication). The results do not support an important role of trace elements in the antidiabetic effect of the studied plants.

The nutritional composition of *nfuma*, a traditional flour obtained from the fruits of *Strychnos madagascariensis* (a tree from tropical and sub-tropical Africa), as prepared by populations in Mozambique, was evaluated by Chemane et al. [2]. The authors determined the content of sugar, amino acids, vitamin E, carotenoids, macrominerals (Ca, Mg, Na, K) and a wide panel of trace elements (Fe, Li, Be, Al, V, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Cd, Cs, Ba, Tl, Pb, Bi) in *nfuma* from four districts of Mozambique, and evaluated its nutritional adequacy, according to current nutritional recommendations. Safety issues, related to possible exposure to potentially toxic trace elements, were also addressed.

The study conducted by Bielecka et al. [3] aimed to determine the content of selected macrominerals and essential trace elements (Ca, Mg, Cu, Fe, Mn, Se and Zn) in rice and rice products in order to as assess their importance as source of these nutrients in the adult European population diet. All products studied proved to be a source of Cu, Mn and Se, while most could also be considered a source of Mg and Zn. Not surprisingly, significant differences in the levels of the studied elements were observed between processed and unprocessed products.

The results from six years (2015–2020) of official control of the Cd, Pd and Hg content in imported whole durum wheat (from Australia, Canada, Kazakhstan, Russia, Turkey and the USA) into the Italian market are reported by Pompa et al. [4]. Data are discussed according to seasonality, year and country of origin. The trend observed in other studies at a global level of a decrease in Pb, Cd and Hg levels in recent years was confirmed. However, durum wheat products continue to represent important sources of exposure to Cd and Pb.

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The effect of different food processing (high-pressure, freezing, frozen storage, canning) on the content of macrominerals and a wide panel of trace elements (Na, K, Mg, Ca, Ba, Mn, Fe, Co, Cu, Cd, Sn, Pb, P, As, S, Se) in brine-canned mackerel (*Scomber colias*) was studied by Prego et al. [5]. Content changes are tentatively explained.

In another work, by Garcia-Galdeano et al. [6], the content of Zn, Cu and Fe in several dehydrated aromatic plants (thyme, rosemary, cloves, oregano and basil) was studied, as well as their microbiological quality (assessed by plate counts of L. monocytogenes and other foodborne pathogens). The growth of several of the foodborne pathogens tested was positively correlated with the levels of Zn, Cu and Fe, which the authors attributed to the fact that those trace elements could act as a "growth factor".

Dahl et al. [7] studied the effect of different food processing (boiling, pan-frying, oven-baking) on the iodine and mercury content of Atlantic cod (*Cadus morhua*). Boiling has been shown to significantly decrease the iodine content (approx. 10–20%), so the authors suggest that this be specified in food composition databases as it may influence the estimation of the actual iodine intake [7].

Lastly, a mini review summarizes recent developments on the use of metallic nanoparticles in the food science and technology sector, including toxicity/biosafety and regulatory issues [8].

Overall, the papers published in this Special Issue highlight the importance and need for further research on trace elements in the food sector. As guest editors, we sincerely hope that readers will find it interesting and informative, and we thank all authors for their highly qualified contributions.

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Review

Metallic Nanoparticles in the Food Sector: A Mini-Review

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Abstract: Nanomaterials, and in particular metallic nanoparticles (MNPs), have significantly contributed to the production of healthier, safer, and higher-quality foods and food packaging with special properties, such as greater mechanical strength, improved gas barrier capacity, increased water repellency and ability to inhibit microbial contamination, ensuring higher quality and longer product shelf life. MNPs can also be incorporated into chemical and biological sensors, enabling the design of fast and sensitive monitoring devices to assess food quality, from freshness to detection of allergens, food-borne pathogens or toxins. This review summarizes recent developments in the use of MNPs in the field of food science and technology. Additionally, a brief overview of MNP synthesis and characterization techniques is provided, as well as of the toxicity, biosafety and regulatory issues of MNPs in the agricultural, feed and food sectors.

Keywords: metallic nanoparticles (MNPs); food; food packaging; food analysis

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1. Introduction

Nanotechnology is the branch of science and engineering that deals with the preparation of nano-size particles, i.e., particles with dimensions from 1–100 nm, using various synthesis strategies that allow obtaining particles with different structures and sizes [1]. With advances in nanotechnology, novel materials (nanomaterials) with peculiar and improved properties (compared to those of atoms, molecules and bulk materials), attributed to their high surface-to-volume ratio (35–45% higher compared to atoms), have progressively emerged [2]. Nanoparticles (NPs) have both solute and separate particle phase properties [3]. The unique extrinsic property of NP of having a large specific surface area is an important feature that contributes to some particular characteristics, namely strong surface reactivity [1,4].

In the last decades, due to their particular properties, NP have received great attention in the most diverse areas, from optical, electronic and medical applications to their use as sensors and catalysts, being used in various fields such as physics, organic and inorganic chemistry, molecular biology, and medicine [1,5,6]. The use of NP in the food sector is relatively more recent, but the special characteristics of mechanical resistance, diffusivity, optical properties and solubility of NP have also led to their introduction in all stages of the food sector chain: production, processing, packaging, transport and storage, with notable impacts on food quality and safety [7,8].

Metallic (or metal) nanoparticles (s) constitute a special and particularly valuable group of NP. Their physicochemical properties depend mainly on the (relatively) free surface electrons, and they present unique characteristics such as high surface energies, high plasmon excitation efficiencies and a variety of unusual and interesting optical properties [9,10]. The use of MNPs in food technology and industry plays a key role in protecting, preserving and extending the shelf life of food [1,10–12]. In particular, the use of MNPs has allowed the improvement of food packaging characteristics, such as mechanical properties,

permeability to water vapor and antibacterial activity [13,14], allowing not only the maintenance of freshness and increases in the shelf life of products, but even the production of safer and more ecologically sound (degradable) food packaging [8]. Additionally, MNPs allowed the development of new devices, namely nanosensors [13,14], and methods for food analysis [8].

The increasing use of MNPs has naturally raised environmental and ecological concerns. One answer to these concerns is the synthesis of metallic nanocomposites (consisting of several nanomaterials entrapped in a bulk material) starting from natural raw materials. In this synthesis strategy, natural biopolymers (e.g., starch, agar, gelatin, chitosan and cellulose [11]) are combined with one or more different types of MNP, and act not only as vehicles for the particles, but also give rise to hybrid systems with specific characteristics, such as large surface areas, ordered crystalline structures and highly regularized pores [11,15,16]. This new approach has been widely explored in recent years due to its environmental friendliness, easy processing and the particularly suitable mechanical and barrier characteristics of the resulting materials [17].

Metal nanotechnology applied to the food sector is, therefore, a clear example of an emerging technology that can have both positive and negative impacts. Its applications are ubiquitous, from agriculture to food processing, packaging and storage, including laboratory quality control. Naturally, it also raises safety concerns, especially regarding its potential effects on human health. A thorough knowledge of the safety and proper risk assessment of the use of MNPs in food processing and packaging is, therefore, crucial. In this mini-review, we aim to present the latest developments in the use of MNPs in food technology, as well as their impact on food quality and safety.

2. Synthesis and Stabilization of MNPs

One of the biggest limitations related to the use of MNPs is the lack of an effective synthesis process that can lead to homogeneous sizes and shapes, as well as particles with little or no toxicity for both humans and the environment [18]. Different physical and chemical methods are generally used for the preparation and stabilization of MNPs, with the reduction of metal ions in a solution using suitable reducing agents being the most widely used chemical process [18]. Precursor concentration, temperature and nature of reducing agent and solvent are the most critical parameters. The kinetics of the interaction between metallic ions and the reducing agent and the kinetics of the adsorption process of the stabilizing agent with the nascent MNP influence their structure, size uniformity and general physicochemical properties [19].

Several approaches are employed for the preparation of MNPs and can generally be classified as the bottom-up and top-down methods (Figure 1), depending on the starting material [3]. In top-down methods, the bulk material is made into nano-sized particles by reducing the size of the starting material through mechanical milling/grinding (dry, wet), laser ablation or ion sputtering. These methods are easy to perform but inadequate for preparing very small-sized, informal shaped particles, and changes in the surface chemistry and physicochemical characteristics of MNPs are observed [3].

The bottom-up approach is based on the formation of MNPs from smaller entities by joining atoms, molecules or small particles. In this approach, the nanostructured building blocks of the nanoparticles are previously formed and then assembled to produce the final MNP [3]. Examples of bottom-up methods are solid state, liquid state, gas phase and biological methods, as well as electrodeposition or supercritical fluid precipitation processes and ultrasound or microwave-assisted techniques [3]. Biological processes to synthesize nanoparticles are considered simpler, cheaper and more ecologically sound compared to conventional chemical methods [18,20]. For this biogenic or green synthesis, fungi, actinomycetes, algae and even higher plants have been used and have shown themselves as potential nanofactories, with high cost-effectiveness and respect for the environment [20]. Nanoparticles produced by biological processes tend to have greater catalytic reactivity and a greater specific surface area due to improved contact between the reducing enzymes

and the metal ion, but biological processes are slower, and the size and shape of the formed nanoparticles are more heterogeneous [21].

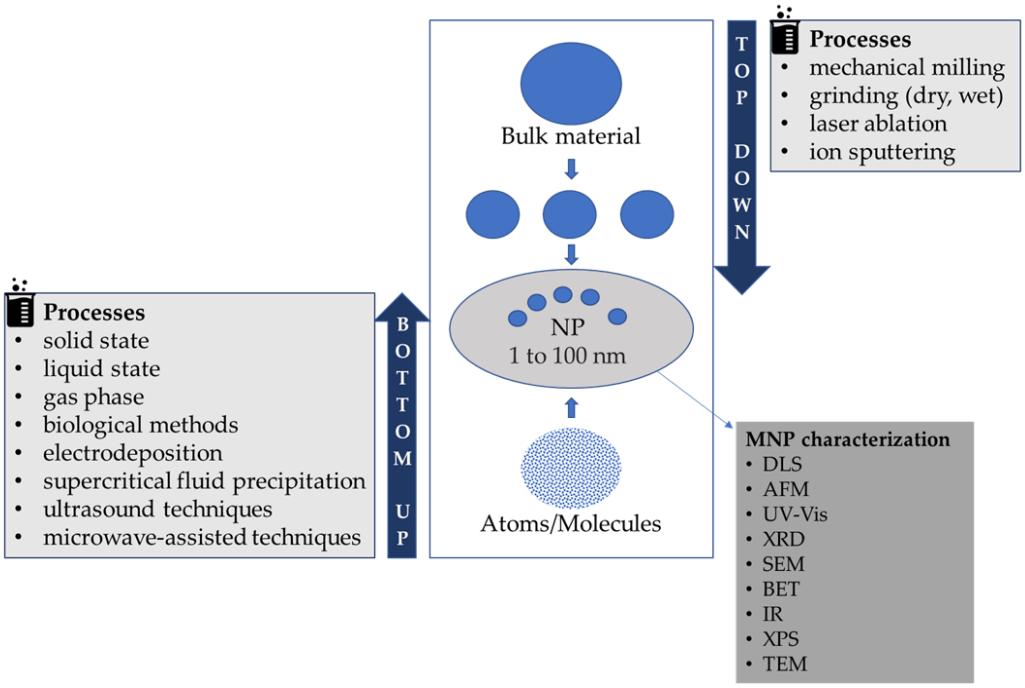

Figure 1. Flowchart representation of MNP preparation and characterization. DLS: dynamic light scattering, AFM: atomic force microscopy, UV-Vis: ultraviolet-visible spectroscopy, XRD: X-ray diffraction, SEM: scanning electron microscopy, BET: Brunauer–Emmett–Teller method, IR: infrared spectroscopy, XPS: X-ray photoelectron spectroscopy, TEM: transmission electron microscopy.

MNPs are commonly stabilized through the adsorption of high-molecular compounds, which form a dispersant layer around the particle surface and prevent their aggregation/coagulation [22]. These "capping" agents can significantly change the physicochemical and biological characteristics of an MNP [23].

Several methods and techniques are available for evaluating the size and physicochemical properties of MNPs, such as dynamic light scattering (DLS), the Brunauer–Emmett–Teller (BET) method, atomic force microscopy (AFM), infrared and UV-Vis spectrophotometry, X-ray diffraction (XRD) and X-ray photoelectron spectroscopy (XPS), scanning electron microscopy (SEM) and transmission electron microscopy (TEM) [6].

3. Uses of MNPs in the Food Sector

Most applications of MNPs in agriculture and the food industry are focused on improving the organoleptic properties of foods (taste, color, texture), increased nutrient absorption, targeted delivery of nutrients and bioactive compounds, stabilization of active ingredients and antimicrobial action against foodborne pathogens. Many are related to packaging innovations to preserve product quality and improve shelf life. MNPs have also been used as sensors to monitor food quality and safety (Figure 2) [24].

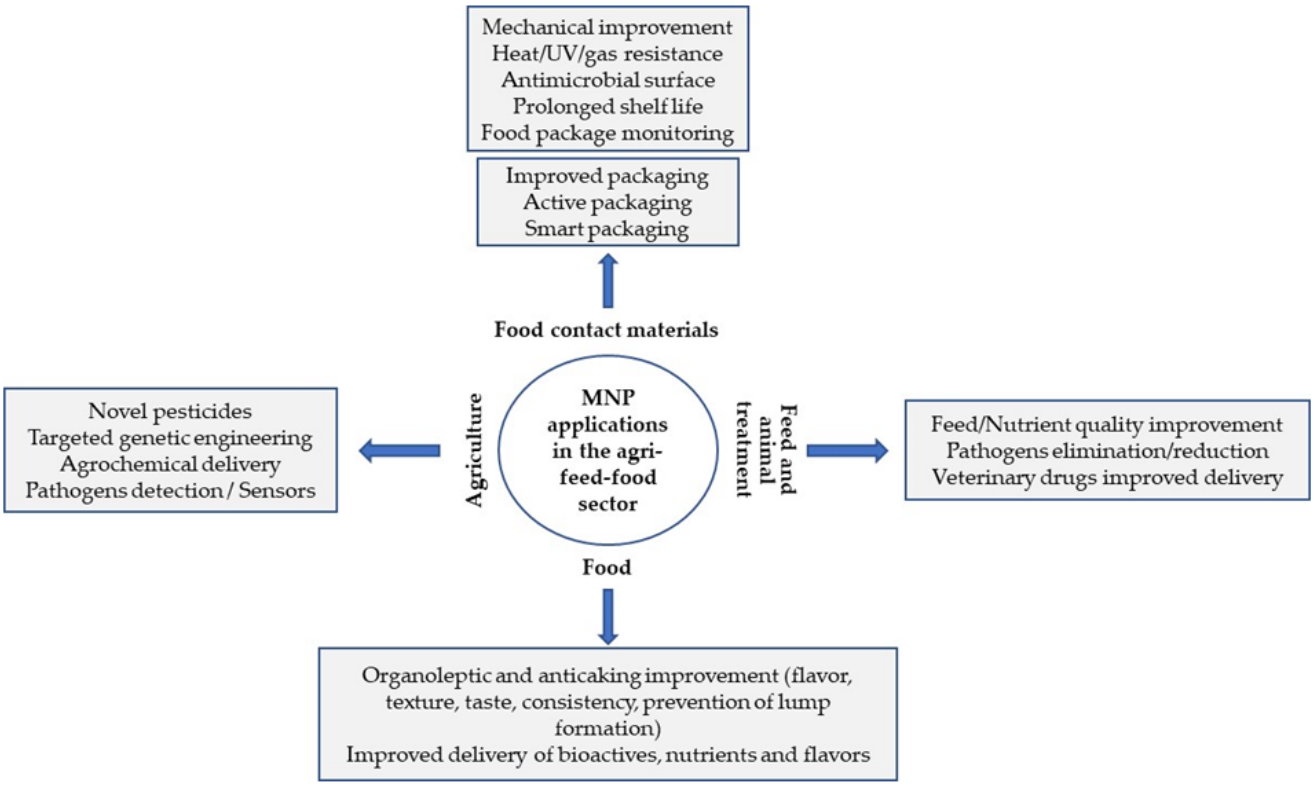

Figure 2. Pictorial representation of some of the main applications of nanotechnology in different agricultural and food sectors (adapted from [25]).

Briefly, MNPs are used to:

- Develop antimicrobial agents with the potential to improve the shelf life of foods and prevent microbial growth. MNPs can destroy microbial cells through different mechanisms and have the potential to inhibit biofilm development [26]. Antimicrobial activity can result from MNP adsorption on the cell wall, destruction of the cell membrane by free radicals, induction of intracellular release of reactive oxygen species (ROS), interaction of metal ions with cell respiratory enzymes and interaction with DNA and proteins [27,28]. This antimicrobial activity depends on the MNP synthesis method, size, shape and type, and nature of the capping agents [23].
- Develop active, smart or biodegradable packaging with increased UV protection and antimicrobial activity, enhanced thermal, hydrophobicity (reduced water vapor permeability) and oxygen barrier properties; the ability to change product color; enhanced radical and oxygen scavenging activities; improved mechanical properties (tensile strength, film thickness and transparency, barrier properties), etc. [8]. Improved food packaging based on functional nanomaterials can be classified into four different categories: physically improved packaging (improved mechanical strength, temperature and moisture stability, gas barrier functions, flexibility and durability); biochemically improved packaging (improved biodegradability, edibility, biocompatibility, low-waste and eco-friendly features); improved packaging with active functions (effect on packaged foods with regard to taste, freshness and shelf life); improved packaging with smart functions (e.g., nanosensors to monitor food conditions such as oxygen levels, freshness and the presence of pathogens) [29].
- Develop MNP-based sensors useful for detecting food contaminants, particularly microbiological pathogens [8,30].

The main current uses of MNPs in the food sector are listed in Table 1.

Table 1. Main uses of metallic nanoparticles (MNPs) in the food sector.

MNP	Application	Film/Analyte/Food	Ref.
Au-NP	SERS sensor	Bisphenol/milk	[31]
	Electrochemical sensor	Ca^{2+}/meat	[32]
	Electrochemical biosensor	Bisphenol A/waters	[33]
	Rapid detection of single or multiple foodborne pathogens	Foodborne pathogens	[34]
	SPR with Au-NPs	–	[35]
	Temperature indicator	Chitosan-capped Au-NPs/frozen products	[36]
	SPR biosensor	Aflatoxin B1	[37]
	SERS active sandwich immunoassay	*E. coli*	[38]
	Filter paper-based SERS substrates	Black phosphorus-Au/*S. aureus*, *L. monocytogenes* and *E. coli*	[39]
	Isothermal RPA detection	*Salmonella* detection/milk	[40]
	Colorimetric sensor	*hlyA* gene and genomic DNA of *Listeria monocytogenes*	[41]
	Aptasensor	*C. jejuni* and *C. coli*/chicken carcass	[42]
	Polymerase chain assay colorimetric sensor	Emetic *Bacillus cereus*/milk	[43]
	Portable plasmonic biosensor	Melamine/infant formula	[44]
	Colloidal Au immunochromatographic strip	Simultaneous detection of *S. boydii* + *E. coli*	[45]
	Paper sensor	*Listeria* spp./milk	[46]
	SERS-based aptasensor	Kanamycin residue/milk	[47]
	Immunochromatographic sensor	26 sulfonamides/commercial honey	[48]
	Low-fouling SPR biosensor	*E. coli* and *Salmonella* spp./cucumber and hamburger	[49]
	Cuvette-type localized SPR optical biosensor	Melamine/infant formulas	[50]
	POC biosensors	Food allergens	[51]
	Optical sensor	Biogenic amines/poultry meat	[52]
Ag-NP	Films with enhanced antibacterial and migration properties	PP-Ag nanocomposite	[53]
	Biodegradable food packaging	Chitosan/gelatin/Ag-NP composites/ carrot pieces	[54]
	Antimicrobial films	LDPE/Ag-NPs	[55]
	Edible coatings (thin layers of material on the product surface)	Ag-chitosan nanocomposites into chitosan coatings/fresh-cut melon	[56]
	Combined use of gamma irradiation and PE/Ag-NP films	PP/Ag-NPs/fresh bottom mushroom	[57]
	Multifunctional packaging	Chitosan coated PE films (lecithin-liposomes/laurel essential oil/Ag-NPs)/pork	[58]
	Active nanocomposite packaging film	PLA/Ag-NPs/strawberries	[59]
	Physico-mechanical and antimicrobial edible films	Tragacanth/HPMC/bees-wax/Ag-NPs	[60]
	Coating films	Guar gum-Ag coatings/coated kinnow (*Citrus reticulata* cv. Blanco)	[61]
	Antimicrobial food packaging	PVA/nanocellulose/Ag nanocomposite	[30]
	Packaging	Biodegradable PVA-montmorillonite K10 clay nanocomposite blend films with in situ generated ginger extract mediated Ag-NP pouches/chicken sausages	[62]
	Antimicrobial materials	Hybrid nanomaterials(cellulose/Ag-NPs)	[63]
	Active food packaging: antimicrobial/antioxidant	Cellulose acetate/AgNP-organoclay and/or thymol nanobiocomposite films	[64]
	Packaging	Chitosan based nanocomposite films incorporated with biogenic Ag-NPs	[65]
	Packaging	Carrageenan/Ag-NP/laponite nanocomposite coated on oxygen plasma surface–modified PP film	[66]
	Packaging	*A. flavus*/mildew and storage of rice	[67]

Table 1. *Cont.*

MNP	Application	Film/Analyte/Food	Ref.
	Packaging	Nano-cellulose composite films/grape seed extracts/Ag-NPs (antimicrobial activity against *E. coli* and *S. aureus* + strong antioxidant activity)	[68]
	Packaging	Cellulosic packets impregnated with Ag-NPs/*Aeromonas* sp. isolated from rotten vegetables (tomatoes and cabbage)	[69]
	Colorimetric assay based on Ag-NPs	Melamine/milk	[70]
	Colorimetric sensor	Ag-NP solution synthesized using culture supernatant *B. subtilis*/volatile compounds released during the deterioration of *Musa acuminata* (banana)	[71]
	Colorimetric sensor	Ag-based nanomaterial/onion postharvest spoilage	[72]
	Packaging	Chitosan-Ag-NPs/minced meat	[73]
	Packaging	Agar film containing nanoAg conjugate (*Cymbopogon citratus* extract/nisin/Ag)/*L. monocytogenes*, *S. aureus*, *P. fluorescens*, *A. niger* and *F. moniliforme*	[74]
	Food spoilage detection and post-harvest spoilage	Cysteine and histidine incorporated Ag-NP lactic acid/fresh milk	[75]
Cu-NP	Antibacterial surfaces	Antibacterial effect of Cu-polymer nanocomposites	[76]
	Packaging	Biodegradable HPMC matrix/Cu-NPs/ *S. aureus*, *S. epidermidis*, *B. cereus*, *E. coli*, *E. faecalis*, *Salmonella spp.*, *P. aeruginosa*/meat	[77]
	Packaging	Chitosan/soy protein isolate nanocomposite film	[78]
	Amperometric paper sensor	Carbohydrates/soft drinks	[79]
	Electrochemical biosensor	Organophosphorus pesticides (chlorpyrifos, fenthion and methylparathion)/cabbage and spinach extract	[80]
	Electrochemical sensor	Malathion/vegetable extracts	[81]
	Active biodegradable film	Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) nanocomposites/CuO-NPs/ *S. enterica*, *L. monocytogenes* and murine norovirus	[82]
Zn-NP	Active food packaging material	Fish protein isolate and fish skin gelatin/ZnO-NPs	[83]
	Packaging	PLA/ZnO biocomposite films	[84]
	Antimicrobial packaging	PLA/ZnO nanocomposite coated paper	[85]
	Biodegradable film	Bionanocomposites of chitosan/ZnO-NPs/apple peels, fresh poultry meat	[86]
	Packaging	Gelatin/ZnO-NP nanocomposite film/ foodborne pathogenic bacteria	[87]
	Packaging	Nanocomposite film of chitosan-ZnO-cellulose/nisin/cheese	[88]
	Packaging	Hybrid nanocellulose-ZnO-NP-based composites	[89]
	Antimicrobial packaging	Chitosan/ZnO coated on PE film	[90]
	Biodegradable food packaging	Soybean protein/ZnO film	[91]
	Food packaging	Chitosan/ZnO antimicrobial pouches/raw meat	[92]
	Food packaging	Ziziphora clinopodioides essential oil/apple peel extract/ZnO-NPs/*Listeria monocytogenes*/sauced silver carp fillet	[93]
	Food packaging	ZnO nanorods/clove essential oil/Type B gelatin composite films/*L. monocytogenes* + *Salmonella* Typhimurium/shrimps	[94]
	Intelligent and active films	Cellulose modified with polypyrrole/ZnO/microbial load chicken thigh	[95]
	Multifunctional bionanocomposite films	Konjac glucomannan/chitosan/ZnO/mulberry anthocyanin extract/*E. coli* and *S. aureus*	[96]
	Coating	Chitosan/ZnO-NPs/microbial growth on fresh-cut papaya	[97]

Table 1. *Cont.*

MNP	Application	Film/Analyte/Food	Ref.
Ti-NP	Active and smart packaging	Chitosan-TiO_2 composite film/antimicrobial activity against *E. coli*, *S. aureus*, *C.albicans*, *A. niger*/red grapes	[98]
	Coating film	TiO_2-NPs/chitosan	[99]
	Edible films and coatings	Whey protein nanofibrils/glycerol/TiO_2/ *L. monocytogenes*, *S. aureus*, *S. enteritidis*, *E. coli/meat*	[100]
	Packaging	Chitosan film/Cymbopogon citratus essential oil/TiO_2-NPs/minced meat	[101]
	UV absorbent film	Gelatin/agar bilayer film and nanocomposites/TiO_2-NPs/fish oil	[102]
	Packaging	TiO_2-NPs/PLA/several bacteria strains	[103]
	Active and smart packaging	PAN/TiO_2 nanofibers/tomato fruit-ripening test	[104]
	Film	Whey protein/cellulose nanofiber/nanocomposite films/TiO_2/rosemary essential oil/foodborne bacteria/lamb meat	[105]
	Food packaging	PLA/TiO_2 composites	[106]
	Amperometric sensor	Graphene/TiO_2 nanocomposite/hypoxanthine/meat freshness evaluation	[107]
	Packaging	PET/TiO_2-NPs/ethylene glycol migration	[108]
	Active and smart packaging	PAN/nanofibers/postharvest ripening of bananas	[109]
	Active food packaging (ethylene scavenging + antimicrobial activity)	Chitosan/TiO_2 nanocomposite film/*S. aureus*, *E.coli*, *Salmonella* Typhimurium, *P. aeruginosa*, *Aspergillus* and *Penicillium*	[110]
	Biodegradable food packaging	Starch/TiO_2 bionanocomposite	[111]
	Biodegradable food packaging	Chitosan/PVA/Ti-NPs/olive oils	[112]
	Active packaging	Chitosan- TiO_2 nanocomposite film/tomato storage shelf life	[113]
	Packaging	Chitosan/PVA/skimmed milk acid coagulated cheese (Karish)	[114]
	Edible coating	CMC/gum arabic/gelatin/garlic extract/TiO_2-NPs/Nile tilapia fish fillets	[115]
Other single metal MNPs	Optical biosensor	Single-layer MnO_2 nanosheets/ascorbic acid/fresh oranges and orange juice	[116]
	Packaging	Chitosan nanocomposite thin films/MgO	[117]
	Active and smart packaging	Nanoscale O_2 scavengers (Fe particles)	[118]
	Active and smart packaging	O_2 scavenging films made of PHB/Pd-NPs	[119]
	Active and smart packaging	Nano zeolite-$Mo_4{}^{2-}$/avocado ripeness indicator	[120]
	Flexible antioxidant packaging (radical scavenging ability)	Embedded Se-NPs/multi-layer plastic/preservation of packages/hazelnuts, walnuts, potato chips	[121]
	Electrochemical biosensor	Graphene-based electrode/nano/microstructured Pt-NPs/phosphonate organophosphates	[122]
	Magnetic solid phase extraction HPLC-DAD	Fe_3O_4 magnetic NPs/multi-walled carbon nanotubes and nanodiamonds/vit.B12/milk-based infant formula, orange and peach juice, meat, salami, powder milk	[123]
	Magnetic solid phase extraction FAAS	Fe_3O_4 -sodium dodecyl sulfate-carbazone/ Cd/green tea. Lettuce, ginseng, rice, spice and carrot	[124]

Table 1. *Cont.*

MNP	Application	Film/Analyte/Food	Ref.
Mixed (bi-/ternary) MNPs	Nanocomposite films	ZnO/CuO-NPs/poly-ε-caprolactone/terephthalic acid	[125]
	Packaging	Hybrid nanomaterials: Ag-NPs, CuO-NPs, ZnO-NPs/cellulose regenerated from cotton linter and microcrystalline cellulose	[63]
	Packaging	Starch-based nanocomposite films: single or combined Ag, ZnO and CuO-NPs/*E. coli* and *S. aureus*	[126]
	Packaging films	Chitin/ZnO/Ag-NPs/CMC/Gram(+) and Gram(−) bacteria	[127]
	Degradable biopolymer nanocomposite	ZnO/Ag nanocomposite/Thymus vulgaris leaf extract/PHB-co-3-hydroxyvalerate)-chitosan/poultries	[128]
	Films	Furcellaran/gelatin/Se-Ag-NPs/*S. aureus,* Multi Resistant *S. aureus* and *E. coli*/ kiwi (*Actinidia arguta*) storage	[129]
	Active packaging	Combination of high-pressure treatment, steak margination and MNPs	[130]
		LDPE/Ag and ZnO-NPs/beef color and shear stress	
	Film	SiO_2/carbon/Ag ternary hybrid polymeric composite/*S. enteritidis*	[131]
	Packaging films	PLA/bergamot essential oils (BEO) or PLA/BEO/nano-TiO_2 or PLA/BEO/nano-TiO_2 + nano-Ag/mangoes	[132]
	Packaging films	LDPE incorporating Ag/CuO/ZnO-NPs/ coliform/ultra-filtrated cheese	[133]
	Electrochemiluminescent immunoassay	Au nanorods functionalized graphene oxide and Pd/Au core-shell nanocrystallines/tomato and chili sauce and powder	[134]
	Active food packaging	PLA films/Ag/Cu-NPs/cinnamon essential oil/chicken meat	[135]
	Nanocomposite films	Ag/Cu and ZnO reinforced PLA	[136]
	Nanocomposite films	Ag/Cu agar-based/*L. monocytogenes*, *Salmonella enterica* serovar Typhimurium	[137]
	Active food packaging material	Ag/Cu guar gum nanocomposite films	[138]
	Packaging film	Ag/TiO_2/PLA/*E. coli*, *L. monocytogenes*	[139]
	Packaging	PLA/TiO_2 and PLA/TiO_2/Ag composite films/cottage cheese samples	[140]

SERS: surface-enhanced Raman spectroscopy; SPR: surface plasmon resonance; RPA: recombinase polymerase amplification; POC: Point of care; PP: polypropylene; LDPE: low-density polyethylene; PE: polyethylene; PLA: polylactic acid; HPMC: hydroxypropylmethylcellulose; PVA: polyvinylalcohol; PAN: polyacrylonitrile; PET: polyethylene terephthalate; CMC: carboxymethyl cellulose; PHB: poly-3-hydroxybutyrate.

3.1. Gold Nanoparticles (Au-NPs)

Au-NPs have received considerable interest in the food industry (Table 1) due to their potential antibacterial activity, inert and nontoxic nature, and oxidative catalytic properties, even though the antibacterial effects of AuNPs are a controversial topic [141]. However, the use of Au-NPs in the development of sensors for food contaminants has been shown to be the most promising application, due to their good characteristics regarding reactivity, selectivity and sensitivity.

Pissuwan et al. [34] recently reviewed the use of Au-NPs in the detection of foodborne pathogens. Several Au-NP-based procedures were identified, including liquid-phase colorimetric assays, refractometric sensing, surface-enhanced Raman scattering (SERS) and immunochromatographic or electrochemical techniques, among others. Another recent review highlighted the latest advances in Au-NP-based biosensors with a focus on fast, low-cost portable biosensors, allowing for on-site assessment of food safety [142]. Progress

specifically in the optical detection of pathogenic bacteria using noble metal nanoparticles, with a focus on colorimetric, SERS and fluorescence assays, was also recently reviewed [143]. Using SERS, a sandwich immunoassay was developed for the detection of *E. coli* using alkaline phosphatase and both spherical gold-coated core-shell Au-NPs and rod-shaped Au-NPs [38]. Additionally, a novel label-free three-dimensional SERS substrate based on black phosphorus-Au filter paper was tested in the rapid detection and discrimination of *S. aureus*, *L. monocytogenes* and *E. coli*, having shown to be a highly sensitive, low-cost alternative to conventional substrates [39].

Other analytical applications of Au-NPs, in addition to food pathogens, have been described. Yang et al. [31] developed an Au-NP-based SERS procedure to improve the detection of bisphenol A residues in milk. Fan et al. [32] developed an electrochemical sensor to determine the Ca^{2+} content in meat. Oxygen plasma-treated graphene was used with Au-NPs. Bhardwaj et al. [37] developed a surface plasmon resonance (SPR)-based nanosensor (chip) with incorporation of Au-NPs for the detection of aflatoxin in wheat samples.

Hybrid metal-polymer matrices, which have distinctive properties as already mentioned, are a new class of materials with applications in food quality control. Mohan et al. [36] tested the effectiveness of nanocomposites containing Au-NPs and chitosan in monitoring the storage conditions of frozen products, where a change in color could indicate an out-of-range temperature.

3.2. Silver Nanoparticles (Ag-NPs)

The role of Ag-NPs in food applications has been extensively reviewed [144]. The exact mechanism of action of Ag-NPs on cells is still unknown, but they can physically interact with the cell surface of different bacteria. Detailed discussion on the possible mechanisms of action of MNPs can be found in [145]. The mechanisms include adhesion to the surface of the bacterial cell wall or membrane, penetration into the cell and disruption of intracellular organelles and biomolecules, induction of oxidative stress and modulation of signal transduction pathways. Adhesion and accumulation of Ag-NPs on the cell surface have been particularly noted in Gram-negative bacteria [28,146].

The antimicrobial effects of Ag-NPs are discussed in several papers (Table 1), and to improve them, the combination of different MNPs or their combination with natural bioactive compounds to obtain biomaterials with increased efficacy has also been tried [147,148]. Packaging materials containing Ag-NPs have been shown to significantly extend the shelf life of products such as rice, potatoes, etc. [67]. Chitosan has been widely used as a polymer in these applications due to its biocompatibility, biodegradability and intrinsic antibacterial properties. Kadam et al. [65] used chitosan-based nanocomposite films impregnated with Ag-NPs biologically synthesized with an extract of Nigella sativa. The film showed a pH-dependent sustained release of Ag-NPs and Ag+ and significant antibacterial activity. Ahmed et al. [135] reported the use of poly(lactic acid) (PLA) biopolymers incorporated with Ag-Cu-NPs and cinnamon oil with significant antimicrobial activity against *Salmonella* Typhimurium, *C. jejuni*, and *L. monocytogenes* in inoculated chicken tissues. Another study, using PLA impregnated with Ag-NPs and titanium dioxide, showed good antimicrobial activity against *E. coli* and *L. monocytogenes* [139]. In another study where a PLA film was used to store rice at high temperature and humidity, due to the presence of Ag^{+} ions, the film showed a cidal effect on *A. flavus* and considerably delayed rice aging [67]. A nanocomposite consisting of a polypropylene (PP) matrix impregnated with Ag-NPs was also tested, and its antimicrobial effectiveness was evaluated against *E. coli* and *S. aureus*. There was a significant enhancement in activity compared to that from isolated, conventional Ag-NPs [53]. In another study [60], a novel trinary nanocomposite film based on tragacanth, hydroxypropylmethylcellulose and beeswax impregnated with Ag-NPs was developed. It proved to be suitable for use in food packaging, as it showed a dose-dependent inhibitory effect against *B. cereus*, *S. aureus*, *S. pneumoniae*, *L. monocytogenes*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *Salmonella* Typhimurium. Olmos et al. [55] studied the effectiveness of

low-density polyethylene-Ag nanocomposites in preserving food against biofilm-forming *E. coli*, and they proved useful for both storage and general-purpose containers. Other hybrid materials, such as polyvinylalcohol (PVA)/nanocellulose/Ag nanocomposite films, were also tested in packaging applications to protect against methicillin-resistant *S. aureus* and *E. coli* [30]. Blend films of PVA-montmorillonite K10 clay nanocomposite with in situ-generated Ag-NPs (using a ginger extract-mediated synthesis procedure) were used to manufacture biodegradable pouches that showed ability to prevent microbial spoilage of chicken sausages [62]. Other studies reported the development of hybrid nanomaterials obtained by one-pot synthesis of Ag-NPs, CuO-NPs and ZnO-NPs during the regeneration of cellulose from cotton linters and microcrystalline cellulose [63]. Vishnuvarthanan et al. [66] developed a carrageenan/Ag-NP/laponite nanocomposite coated on oxygen plasma surface–modified PP film. Ag-NPs were synthesized using extract of *Digitalis purpurea*. Characteristics such as adhesion, mechanical barrier and antimicrobial properties of the nanocomposites were significantly increased, as well as the activity against *E. coli* and *S. aureus*. Dairi et al. [64] studied nanobiocomposite films of Ag-NPs, gelatin-modified montmorillonite nanofiller and thymol, biogenically synthesized using *Curcuma longa* tuber extracts. The films showed antibacterial, antifungal and antioxidant activities, with the ability to extend the shelf life of fruits. Starch (St)-based nanocomposite films containing single or combined Ag, ZnO and CuO nanoparticles were prepared, and microbial tests showed that St-Ag and St-CuO films had the highest antibacterial activity against *E. coli* and *S. aureus*. Increasing the NP concentration from 1 to 3% (*w*/*w*) increased the antibacterial effect. The combined use of Ag/ZnO/ CuO-NPs in the formulation showed a synergistic effect on the antimicrobial and mechanical properties of the films, allowing the dose reduction of each individual MNP [126].

Ag-NPs have also been shown to be very efficient in detecting food spoilage and evaluating post-harvest spoilage of agricultural and horticultural crops. One work described the use of cysteine- and histidine-incorporated Ag-NPs to detect lactic acid in fresh milk by color change [75]. A recent review describes other Ag-NP films that have been applied to various fruit crops such as bananas, tomatoes and kiwis to detect spoilage through color change [13].

3.3. Copper Nanoparticles (Cu-NPs)

Cu-based nanocomposites are particularly interesting because they are less expensive than Ag equivalents. They have been reported to have potential antibacterial activity against a wide range of Gram-positive and Gram-negative bacteria due to Cu ion release, Cu-NP release from nanocomposites, and inhibition of bacterial biofilm formation [11]. A review on the use of Cu-polymer nanocomposites presented several studies showing antimicrobial activity against *S. aureus*, *E. coli*, *S. cerevisiae*, *Streptococcus* spp., and *Pseudomonas* spp. in food [76]. Antimicrobial activity against *S. aureus*, *S. epidermidis*, *B. cereus*, *E. coli*, *E. faecalis*, *Salmonella* spp. and *P. aeruginosa* in the packaging and preservation of food products such as meat was evaluated using a biodegradable hydroxypropylmethylcellulose matrix impregnated with Cu-NPs [77]. Varaprasad et al. [125] produced metal-oxide polymer nanocomposite films using biodegradable poly-ε-caprolactone, polyethylene terephthalate (PET; discarded oil bottles) and ZnO-CuO nanoparticles. Metal oxide-polymer nanocomposite films have demonstrated excellent mechanical properties and have been reported to be functional in household packaging. Works by Arfat et al. [137,138] evaluated Ag-Cu alloy nanoparticle composite films based on agar or guar gum. In addition to showing good mechanical strength, UV light protection and oxygen barrier efficacy, the films showed antibacterial activity against *L. monocytogenes* and *Salmonella* Typhimurium, proving to have high potential as films usable in active food packaging. Ahmed et al. [136] prepared plasticized PLA-based nanocomposite films incorporating polyethylene glycol and Ag-Cu alloy or ZnO nanoparticles. Later, the same authors prepared plasticized PLA composite films impregnated with Ag-Cu-NPs and cinnamon essential oil, as already mentioned. Bacterial growth was remarkably reduced when a film containing

50% cinnamon essential oil was used in the packaging of chicken meat [135]. Active biodegradable poly(3-hydroxybutyrate-co-3-hydroxyvalerate) melt mixed nanocomposites and bilayer structures containing CuO-NPs were developed and characterized by Castro Mayorga et al. [82]. The products exhibited significant bactericidal and virucidal action against foodborne pathogens *S. enterica*, *L. monocytogenes* and murine norovirus.

3.4. Zinc Nanoparticles (Zn-NPs)

Transition metal oxide nanomaterials have shown high antibacterial activity and, among them, ZnO-NPs showed clear superiority. This is due to a distinctive electronic configuration, which, in particular, causes them to lead to the formation of ROS following light absorption [149].

The mechanisms responsible for the antimicrobial activity of ZnO-NPs are not fully understood, but it is believed that it will depend on the action of released Zn^{2+} ions (with antimicrobial activity) [145,149] and that the contact of the nanoparticles with the bacterial surface is capable of causing the formation of electrostatic forces that damage the cell membrane [150]. However, the most plausible mechanism is the formation of reactive oxygen species, including hydrogen peroxide, although it is not entirely clear how these species are produced [28,145,149–152].

Different polymers such as chitosan, poly(3-hydroxybutyrate), poly(butylene adipate-co-terephthalate), low-density polyethylene, semolina flour and bovine skin gelatin have been used to produce ZnO-NP-based nanocomposites, and it has been shown that the incorporation of ZnO-NPs in polymeric films makes them fire resistant, lighter, thermally and mechanically improved and less permeable to moisture and gases, thus more suitable for food packaging [15]. Marra et al. [84] proved that a PLA film with 5% (*w*/*w*) ZnO is suitable for use in food packaging, having good tensile properties, lower permeability to O_2 and CO_2 and excellent antimicrobial activity against *E. coli.* ZnO nanoparticles were used by Zhang et al. [85] to develop a paper-based packaging material coated with a ZnO-PLA layer, and it showed efficacy in inactivating both *E. coli* and *S. aureus*.

A study described the production of sustainable, low-cost, antimicrobially active bio-nanocomposites consisting of ZnO-NPs incorporated in chitosan, obtained from food industry by-products (apple peel). Their hydrophobic, mechanical, optical and barrier properties were characterized. The films proved to be efficient in extending the shelf-life of fresh poultry meat, and the incorporation of ZnO-NPs enhanced their antimicrobial and antioxidant properties [86]. A gelatin/ZnO-NP nanocomposite film was evaluated, and its antibacterial activity was confirmed, showing it to be more active against Gram-positive than Gram-negative foodborne pathogenic bacteria [87]. In a work by Arfat et al. [83], a nanocomposite film consisting of fish protein isolate plus fish skin gelatin and ZnO-NPs was characterized, and also exhibited strong antibacterial activity. A nanocomposite film consisting of chitosan-ZnO-cellulose containing nisin revealed potent antibacterial activity and could be used for cheese packaging [88]. Nanocellulose has proven to be a promising natural material for the production of hybrid nanocellulose-ZnO-NP-based composites with excellent mechanical, UV protection and antibacterial properties, suitable for use in food packaging [89]. Another study explored the natural properties of chitin combined with ZnO/Ag-NPs incorporated into carboxymethylcellulose, and the hybrid material allowed obtaining films with antibacterial activity against both Gram-positive and Gram-negative bacteria [127]. The incorporation of ZnO-Ag, with *Thymus vulgaris* leaf extract as a stabilizer, into poly(3- hydroxybutyrate-co-3-hydroxyvalerate)-chitosan yielded a novel biodegradable biopolymer nanocomposite. It presented good mechanical characteristics and strong antimicrobial activity, which allowed an improvement in the shelf life of poultry meat, suggesting a potential for replacing the traditional petrochemical-based polymers currently used [128].

The antimicrobial properties of chitosan-ZnO nanocomposite coatings on polyethylene (PE) films were studied, and the complete inactivation of food pathogens *S. enterica*, *E. coli* and *S. aureus* was observed [90]. Chitosan-ZnO nanocomposite portable pouches were

developed as smart packaging. A one-pot procedure was adopted for the preparation of the films, which exhibited excellent antimicrobial activity in the packaging of raw meat [92].

3.5. Titanium Nanoparticles (Ti-NPs)

Titanium nanostructures have also shown promise with regard to food processing and safety due to their low cost, chemical stability, photocatalytic activity and biocompatibility, in addition to their antimicrobial activity [99]. The antimicrobial activity is probably due to the generation of reactive oxygen species (ROS) [99,145]. Recently, most attention has focused on the combination of TiO_2-NP and chitosan. Youssef et al. [153] used a chitosan/PVA/TiO_2-NP nanocomposite as packaging material for soft white cheese, achieving improved shelf life and reduced bacteria, yeast and mold counts compared to the control. Another chitosan film was prepared with *Cymbopogon citratus* essential oil (CCEO) and TiO_2-NPs, and minced meat was packaged with the developed films and stored at 4 °C for 10 days. The incorporation of TiO_2-NPs increased the water vapor permeability and the tensile strength, while the addition of CCEO extended the meat shelf life [101]. Another report described the effect of a gelatin/agar bilayer film and nanocomposites containing different concentrations of TiO_2-NPs on the oxidative stability of fish oil. Their ultraviolet and oxygen barrier properties allowed minimum photo-oxidation and auto-oxidation during the storage period [102]. A recent review focused on PLA/TiO_2 composites, detailing the approaches used to increase the TiO_2 dispersion and properties of the composites, and concluded that they are promising materials for food packaging applications [106]. A study in which TiO_2-NPs were added to PLA matrices showed good results against a wide variety of bacterial strains [103]. Concerns associated with migration from PLA/TiO_2 and PLA/TiO_2/Ag composite films were the aim of a study using cottage cheese samples, and their safety as antimicrobial food packaging films was confirmed [140].

The effectiveness of edible nanocomposite films consisting of whey protein isolate/cellulose nanofiber containing TiO_2 and a rosemary essential oil in preserving the microbial and sensory quality of lamb meat during storage at 4 °C was evaluated, and greater inhibition was observed against Gram-positive versus Gram-negative bacteria [105]. A PE-based nanocomposite packaging material containing attapulgite and Ag, SiO_2 and TiO_2 (200–400 nm) particles was developed and tested in the preservation of mushrooms (*Flammulina velutipes*) during storage at 4 ºC. It showed the ability to regulate oxygen and carbon dioxide levels, eliminate released ethylene (possibly absorbed by nanoparticles) and inhibit microbial growth, proving superiority over normal PE material [154]. The migration of ethylene glycol from PET bottles (neat PET and PET nanocomposites) was investigated in a study using TiO_2-NPs [108]. The migration test (acidic food simulant) showed a lower release of ethylene glycol from nanocomposite bottles (0.09 mg/kg after 15 days versus 0.16 mg/kg after 15 days in neat PET bottles).

3.6. Other MNPs

There are some reports on the use of other metals and metal oxide nanoparticles in active food packaging applications and analysis: for example, oxygen scavenging films based on poly(3-hydroxybutyrate) (PHB) impregnated with Pd-NPs [119] and radical scavenging films containing Se-NPs impregnated in a multi-layer plastic packaging material, which extended the shelf life of food products (hazelnuts, walnuts and potato chips), showing antioxidant properties [121]. Iron NPs and especially magnetic Fe-NPs prepared using iron oxides such as magnetite (Fe_3O_4) and its oxidized form, maghemite, have also been used [155]. Magnetic Fe-NPs have been used as colorants and sources of bioavailable iron. Many works report the combination of FeO-NPs with carbon nanotubes [123] or other matrices as extraction sorbents in food analysis [156,157]. Magnetic MNPs have been used for the analysis of inorganic and organic compounds in milk, fruit, oil, cereal-based products, beverages, eggs, cacao, and honey, among others [155–157].

4. Safety and Regulatory Issues

New products and ingredients can pose risks to both human health and the environment. The potential toxicity and the biosafety of MNPs and MNP-containing materials is a major concern in the agricultural and food sectors. Polymers commonly used as films are predominantly composed of environmentally friendly and biocompatible materials. However, MNPs are often considered to have toxic potential, depending on exposure factors. Their possible toxicological effects have been extensively studied by both in vitro and in vivo approaches. Kumar et al. [158] reviewed the toxicological effects of MNPs in different experimental models, such as bacteria, microalgae, zebrafish, crustaceans, fish, rat, mouse, pig, guinea pig, human cell lines, and humans. The effects on organs (liver, kidney, spleen, sperm, neural tissues, liver lysosomes, spleen macrophages, glioblastoma cells, hematoma cells and various mammalian cell lines) were also evaluated. Several studies showed that Ag-NPs induced genotoxicity and cytotoxicity in fish, accumulation in gill tissues, lysosomal destabilization in adults and adverse effects on oyster embryonic development, oxidative stress and p53 protein expression, and induction of apoptosis and oxidative stress in the liver in zebrafish [159–161]. ZnO-NP toxicity has been reported in human cervix carcinoma (HEp-2), human HEK293 hepatocytes, and human bronchial epithelial cell lines, demonstrating that ZnO-NPs were toxic to cells, causing DNA damage, oxidative stress and reduction in cell viability [162]. The toxicity of Ti-NPs was studied by Kiss et al. [163], and they were shown to affect cell differentiation, proliferation, apoptosis and mobility. Bour et al. [164] demonstrated the toxicity of CeO_2-NPs to aquatic environments by inhibiting living systems of different trophic levels. Rajput et al. [165], addressing an important ecotoxicological concern, investigated the phytotoxicity of CuO-NPs in spring barley, one of the most important staple food crops, studying their effects on *H. sativum* grown in a hydroponic system.

It must be stressed that the recommendation of the Organisation for Economic Cooperation and Development (OECD) Council on the Safety Testing and Assessment of Manufactured Nanomaterials (adopted on 19 September 2013) assumes "that the approaches for the testing and assessment of traditional chemicals are in general appropriate for assessing the safety of nanomaterials, but may have to be adapted to the specificities of nanomaterials" [166]. Thus, regulation of nanomaterials and nanotechnology is of utmost importance. In particular, it is imperative to regulate the production, handling and use of nanoparticles and nanomaterials, either by legislation or simply by guidelines and recommendations [167,168]. A critical review on the migration potential of nanoparticles in food contact plastics was provided by [169].

The European legislation most closely related to the use of MNPs in the food sector is shown in Table 2.

Regulation (EC) No 178/2002 (General Food Law Regulation) is the foundation of food and feed law in the European Union (EU). It established the general framework for the development of legislation both at EU and national levels, laying down the general principles and requirements of food law, and the general procedures in matters of food safety, and covers all sectors of the food chain "from farm to fork" (including feed production, primary production, food processing, storage, transport and retail sale). It also established the European Food Safety Authority (EFSA), an agency responsible for scientific advice and support, and created the system for the management of emergencies and crises, including the Rapid Alert System for Food and Feed (RASFF) [170].

"Nanofoods"—comprising "food that has been cultivated, produced, processed or packaged using nanotechnology techniques or tools, or to which manufactured nanomaterials have been added" [171]—are covered by Regulation (EU) 2015/2283 on novel foods. Under this Regulation, food consisting of "engineered nanomaterials" should be considered a novel food, and for the purposes of the Regulation, "engineered nanomaterial" means "any intentionally produced material that has one or more dimensions of the order of 100 nm or less or that is composed of discrete functional parts, either internally or at the surface, many of which have one or more dimensions of the order of 100 nm or less,

including structures, agglomerates or aggregates, which may have a size above the order of 100 nm but retain properties that are characteristic of the nanoscale" [172].

Table 2. European Union legislation relevant to the use of metallic nanoparticles (MNPs) in the food sector.

Law	Aim and Scope
Regulation (EC) No 178/2002 (General Food Law Regulation)	Laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety
Regulation (EU) 2015/2283 (Novel Foods)	Lays down rules for the placing of novel foods on the market within the Union
Regulation (EC) No 1333/2008	Community lists of approved food additives, conditions of use of food additives in foods, and rules on the labelling of food additives
Regulation (EU) No 609/2013	On food intended for infants and young children, food for special medical purposes, and total diet replacements for weight control
Regulation (EC) No 1334/2008	On flavourings and certain food ingredients with flavouring properties
Regulation (EC) No 1332/2008	On food enzymes
Directive 2002/46/EC	On the approximation of the laws of the Member States relating to food supplements
Regulation (EC) No 1925/2006	On the addition of vitamins and minerals and of certain other substances to foods
Regulation (EC) No 1935/2004	On materials and articles intended to come into contact with food
Commission Regulation (EU) No 10/2011	On plastic materials and articles intended to come into contact with food
Commission Regulation (EC) No 450/2009	On active and intelligent materials and articles intended to come into contact with food
Regulation (EU) No 1169/2011	On the provision of food information to consumers
Regulation (EC) No 1924/2006	On nutrition and health claims made on foods

Regulation (EU) No 1169/2011, on the provision of food information to consumers, lays down that "all ingredients present in the form of engineered nanomaterials shall be clearly indicated in the list of ingredients" (with the word "nano" in brackets after the name of the ingredient) [173].

The Food Additives Regulation (Regulation (EC) No 1333/2008) stipulates that when there is a "significant change", namely "a change in particle size, for example through nanotechnology", in an existing food additive, it shall be considered a new additive and must be submitted for evaluation by the EFSA [174]. The same applies to substances under Regulation (EU) No 609/2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control [175].

Commission Regulation (EU) No 10/2011 on plastic materials and articles intended to come into contact with food highlights that nanoparticles ("substances in nanoform") may present different toxicological properties than the same substance in conventional particle size. Therefore, they must necessarily be subject to a specific risk assessment and must only be used after explicit authorization [176]. Idem for Commission Regulation (EC) No 450/2009 covers active and intelligent materials and articles intended to come into contact with food (defined as "materials and articles which monitor the condition of packaged food or the environment surrounding the food") [177].

Very recently (August 2021), the EFSA released two updated guidelines—"Guidance on risk assessment of nanomaterials to be applied in the food and feed chain: human and animal health" [178] and "Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles" [179]—applicable in the regulated areas of food and feed products (novel foods,

food contact materials, food/feed additives, etc.). The first guidance focuses specifically on physicochemical characterization, key parameters to be evaluated, methods and techniques that can be used for the characterization of nanomaterials and their determination in complex matrices, as well as aspects related to exposure assessment and hazard identification and characterization [178]. The second guidance describes the assessment criteria (including solubility and dissolution rates of nanoparticles) for deciding when conventional risk assessment should be complemented with nano-specific considerations [179].

In the U.S., the Food and Drug Administration (FDA) has issued a Guidance for Industry titled "Considering whether an FDA-regulated product involves the application of nanotechnology" [180], which represents the Agency's current thinking on this topic. It applies to all "FDA-regulated products", which includes food substances (including food for animals) and dietary supplements.

In another Guidance for Industry, titled "Assessing the effects of significant manufacturing process changes, including emerging technologies, on the safety and regulatory status of food ingredients and food contact substances, including food ingredients that are color additives" [181], additional guidance is provided regarding food ingredients and food contact substances.

Briefly, FDA regulatory intervention regarding applications of nanotechnology or the use of nanomaterials involves two different approaches [182]:

Where products are subject to mandatory premarket review (e.g., food additives, certain new ingredients in dietary supplements), applicants are required to submit data on product safety. Moreover, the premarket review procedure includes special attention to whether the use of nanomaterials suggests the need for additional data on safety.

Where such mandatory premarket review does not exist (e.g., dietary supplements, food), manufacturers are encouraged to consult with the Agency before bringing their products to market to assess the risks of unintended harm to human or animal health.

5. Final Remarks

MNPs have shown great potential in the food sector, contributing to enhance organoleptic properties and improve packaging and storage conditions for food products through innovative, active and intelligent packaging materials, contributing to increase safety, preserve nutritional value and extend product shelf life. In particular, there has been enormous progress in the development of composite nanomaterials with increased biocompatibility through the use of natural biopolymers and the use of more environmentally friendly synthesis processes.

However, the potential benefits and risks must to be carefully assessed. Strategies for specific biosafety risk assessment and definition of regulatory frameworks are still a challenge that requires extensive research. In particular, innovative tests are needed to assess the potential long-term effects of MNPs on human health and the environment. Furthermore, it is essential to formulate strict regulations regarding their safe use in the food industry and to closely monitor compliance.

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Nutritional Characterization of *Strychnos madagascariensis* Fruit Flour Produced by Mozambican Communities and Evaluation of Its Contribution to Nutrient Adequacy

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Abstract: The indigenous fruit *Strychnos madagascariensis* is usually processed to flour, called *nfuma*, being highly consumed during staple food shortage. This study aimed to evaluate the nutritional composition of *nfuma* and its nutrient adequacy. Flours from four districts of Mozambique were analyzed using AOAC methods for proximate composition, HPLC for sugar, amino acids (AA), vitamin E and carotenoids and ICP-MS and FAAS for minerals. The results showed that *nfuma* stands out for its high content of fat (26.3–27.8%), mainly oleic acid, fiber (>6%), vitamin E (6.7 to 8.0 mg/100 g) and carotenes (2.2 to 2.6 mg/100 g). The main amino acids of *nfuma* protein were Arg, Asp and Glu, and Lys was the limiting one. The mineral composition reveals K (~1200 to 1700 mg/100 g) as the main macromineral followed by Mg > Ca > Na. The main trace element was Mn (~4 mg/100 g) followed by Fe > Zn > Cu > Cr > Co. Aluminum (~3 mg/100 g) was the main non-essential element and Rb, Ni, Sr, Ba, V, Cd were also quantified. Assuming the daily consumption of 50 g, *nfuma* provides 82% of Vitamin A dietary reference value for toddlers, while the consumption of 100 g contributes to 132% and 60% of Mn and vitamin A DRV for adults, respectively. Despite the nutritional advantages of *nfuma*, this flour can be a source of Ni, highlighting the importance of the study of good practices in its preparation to decrease the exposure to non-essential elements.

Keywords: monkey orange; fruit flour; macronutrients; micronutrients; indigenous fruits; estimated daily intake

1. Introduction

In recent years, the increase in knowledge about the protective role of fruits and vegetables has led to an increase in campaigns to promote their consumption for better health. Although fruits and vegetables can be consumed in *natura*, since fresh products are highly perishable, they can be processed to increase their shelf life and maintain (or even improve) their nutritional quality and sensory characteristics [1].

Indigenous fruits have been receiving considerable attention from the scientific community as they can be important contributors to the diet of people in developing countries,

reducing nutritional deficiencies and food insecurity, as well as improving the health and economic status of those populations [2,3]. In addition, they can be exploited by the agro-industry and become a source of income for local communities in the future [4,5]. Indigenous fruits are easily accessible to the most vulnerable people because fruit trees are not farmed and often grow in forests and around homes and fields. However, in African countries, indigenous fruits are still underutilized, while several communities are food insecure and, consequently, malnourished [5–8].

Strychnos spp. (monkey orange) is an indigenous fruit tree known for its edible fruits and drought tolerance. However, it has been labeled as a "lost fruit"—fruit that have potential as food- and cash-crops, but little attention has been paid by scientists, policymakers, and the world at large [8]. Thus, little attention has been paid to its potential commercialization, due to limited knowledge and disseminated information compared to many other exotic fruits [9]. Despite some imprecision in species differentiation, five species are prevalent and most consumed in southern Africa (*S. innocua*, *S. cocculoides*, *S. pungens S. spinosa*, and *S. madagascariensis*). Due to their seasonality and high perishability, traditional fruit processing is a very common practice, in addition to their immediate consumption as fresh fruits, with *S. madagascariensis* and *S. innocua* being processed preferably into dry products [10]. Significant variations in the nutritional composition of these fruits have been reported in the literature, and scarce information is available concerning their processed products [9].

Mozambique has numerous native and exotic fruit species that are important for rural communities' survival in times of food shortages. The *S. madagascariensis* fruit, known in southern Mozambique as "macuácua", can be consumed immediately in natura, but it is usually processed into flour by local communities to increase the stability and shelf life of the fruit. Once harvested, the fruit pulp is first dried under the sun and roasted over a fire, and then ground to produce *nfuma* flour, which is consumed by local communities as a snack or as a complement of staple foods in times of food scarcity. However, there is practically no data on its nutritional value. Thus, this study aimed to evaluate the nutritional composition of *nfuma*, the fruit flour of *S. madagascariensis,* and its adequacy in terms of nutrients in light of current recommendations.

2. Materials and Methods

2.1. Fruit Collection and Flour Preparation

Fruit samples were collected by residents of four different districts (Marracuene, Manhiça, Chókwè and Chicualacuala) in southern Mozambique. A total of 480 fresh fruits, weighing 510 $\pm$ 75 g (n = 12 fruits, randomly selected), were harvested per region (eight randomly selected trees; 15 fruits per tree; three different times), during summer, from October to December. *Nfuma* was traditionally prepared by local residents. Briefly, 120 ripe fruits were broken and the orange pulp with seeds was left to dry in the sun (2 to 4 days), to facilitate the removal of the seeds. Then, the pulp was roasted at about 50–60 °C (temperature measured using a digital thermocouple with a surface probe), on a metal plate under fire, for about 1 h. The dried pulp was then ground into flour using a pestle and mortar, producing about 5 kg of flour. The process was repeated three independent times per region. A schematic description of the sampling methodology is provided in Figure 1.

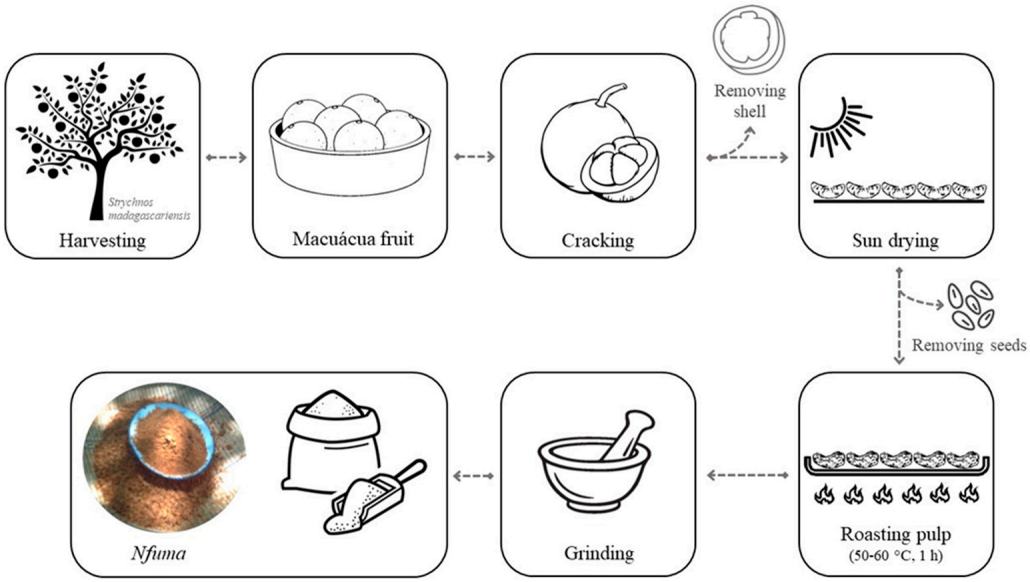

Figure 1. Schematic description of the preparation of *nfuma* by Mozambican communities: from the fruit of *Strychnos madagascariensis* to flour.

2.2. Chemical Analysis

2.2.1. Proximate Analysis

Moisture, crude fat, protein and ash contents were determined according to the Association of Official Analytical Chemists (AOAC) methods 925.09, 920.39, 992.15 and 923.03, respectively [11]. Total dietary fiber was determined by the enzymatic-gravimetric method based on American Association of Cereal Chemists 32–05.01 method and AOAC 985.29 method, according to Martins et al. [12]. Carbohydrates were calculated by the differential method and the energy value was determined based on Regulation (EU) No 1169/2011 [13]. That is, the energy content was calculated from the amount of protein, fat, available carbohydrates and fiber using the factors 17, 37, 17 and 8 kJ per gram (4, 9, 4 and 2 kcal per gram), respectively.

2.2.2. Low Molecular Weight Carbohydrates Determination by HPLC-RI

The low molecular weight carbohydrates (mono and disaccharides) were determined by high-performance liquid chromatography with refractive index detection (HPLC-RI), as described by Santos et al. [14], with some modifications. Briefly, five hundred milligrams of flour were accurately weighed into a centrifuge tube. Prior to sugar extraction, the oil was removed from the flour with the aid of three 5 mL portions of hexane, discarded after centrifugation, and the solid residue was left under a nitrogen stream to evaporate the solvent. The defatted flour was then mixed with 5 mL of ethanol (50% v/v), for sugar extraction. The suspension was stirred for 30 s and the extraction was carried out in an ultrasonic bath (FungiLab, Barcelona, Spain) for 30 min at 50 °C. Thereafter, the mixture was centrifuged at 5000× g at 4 °C for 10 min and 2 mL of the supernatant were left under a nitrogen stream to reduce the ethanol fraction. The final volume was then rigorously adjusted to 2 mL with acetonitrile and allowed to stand for 20 min. The final solution was centrifuged at 5000× g for 10 min at 4 °C and filtered through a 0.22 µm PTFE filter prior to injection.

2.2.3. Fatty Acids Composition by GC-FID

The fatty acid composition of extractable lipids was evaluated as methyl esters derivatives by gas chromatography with flame ionization detection (GC-FID), using alkaline trans-esterification with methanolic potassium hydroxide, as detailed in Regulation EEC 2568/91 [15]. The analysis was performed using a Chrompack CP 9001 gas chromato-

graph (Middelburg, the Netherlands), equipped with a split–splitless injector, a flame ionization detector, an autosampler (Chrompack CP 9050) and a 50 m × 0.25 mm id fused silica capillary column coated with SelectFAME. Helium was used as carrier gas at an internal pressure of 120 kPa. The detector and injector temperatures were 250 and 230 °C, respectively. The results were initially expressed as a relative percentage of each fatty acid methyl ester, without discriminating positional and geometric isomers, calculated by internal normalization of the chromatographic peak areas after standardization of the detector response with the certified reference standard, and converted to the flour mass based on the determined fat content.

Atherogenicity and Thrombogenicity Indexes

The nutritional quality parameters atherogenic index (AI) and thrombogenic index (TI) were calculated according to [16]:

$$\mathrm{AI} = \frac{\mathrm{C12:0} + 4 \times \mathrm{C14:0} + \mathrm{C16:0}}{\mathrm{MUFA} + \mathrm{n3\ PUFA} + \mathrm{n6\ PUFA}} \quad (1)$$

$$\mathrm{TI} = \frac{\mathrm{C14:0} + \mathrm{C16:0} + \mathrm{C18:0}}{0.5 \times \mathrm{MUFA} + 0.5 \times \mathrm{n6\ PUFA} + 3 \times \mathrm{n3\ PUFA} + \frac{\mathrm{n3\ PUFA}}{\mathrm{n6\ PUFA}}} \quad (2)$$

where MUFA and PUFA correspond to monounsaturated and polyunsaturated fatty acids, respectively.

2.2.4. Analysis of Vitamin E and Carotenoids by HPLC-DAD/FLD

The vitamin E and total carotene contents of the lipid extract were determined by normal-phase high-performance liquid chromatography with diode-array and fluorescence detection (HPLC-DAD/FLD), as described in [17]. An exact amount of fat was dissolved in hexane, an appropriate volume of the internal standard solution (tocol) was added, and the mixture was homogenized by stirring. A normal phase silica column (Supelcosil TM LC-SI; 7.5 cm × 3 mm; 3 µm) (Supelco, Bellefonte, PA, USA), conditioned at 25 °C and eluted with a gradient of 1,4-dioxane in hexane at a flow rate of 0.75 mL/min was used. Detection was programmed for excitation at 290 nm and emission at 330 nm for tocols and 450 nm for carotenes. The different vitamin E compounds were identified by comparing retention times with standards, and quantified through individual calibration curves, being expressed in mg of tocopherol/100 g of flour. β-carotene was also quantified based on a calibration curve.

2.2.5. Amino Acid Composition by HPLC-FLD and Assessment of Protein Quality

Amino acids (Asp, Glu, Ser, Hi, Gly, Thr, Arg, Ala, Tyr, Val, Met, Phe, Ile, Leu, Lys, Pro, Trp, Cys, where Asp means aspartic acid/asparagine and Glu means glutamic acid/glutamine) were analyzed by HPLC-FLD, after hydrolysis and derivatization with 9-fluorenylmethyl chloroformate and O-phthaldialdehyde, according to Benhammouche et al. (2021). Briefly, about 100 mg of flour (±3.5 mg of protein) was weighed into a glass crimp vial. An amount of 10 mL of hydrochloric acid solution HCl 6 M containing 0.5% (w/v) phenol were added, sealed and the acid hydrolysis was performed at 110 °C for 24 h. An amount of 1 mL from the resulting hydrolysate was taken and neutralized with NaOH 6 N, and the final volume was made up to 10 mL with borate buffer (0.1 M). An amount of 32 µL of the neutralized solution was mixed with 8 µL of internal standard 250 µM (Norvaline) and 40 µL O-phthaldialdehyde and 20 µL 9-fluorenylmethyl chloroformate were added. Trp was determined separately using alkaline hydrolysis (NaOH 4.2 N, for 18 h at 110 °C). The resulting derivatization products were then subjected to HPLC analysis under the conditions detailed in [18]. Amino acids (AAs) content was reported as mg of AA/g protein.

In order to evaluate the quality of protein in *nfuma*, the essential amino acid profile (EAA) scores (EAAS) and EAA index (EAAI) were calculated using the following equations [19]:

$$\text{EAAS} = \frac{\text{EAA}_{\text{test protein (mg/g)}}}{\text{EAA}_{\text{reference protein (mg/g)}}} \quad (3)$$

$$\text{EAAI} = \sqrt[n]{\text{EAAS 1} \times \text{EAAS 2} \times \text{EAAS 3} \times \text{EAAS n}} \times 100 \quad (4)$$

where n is the number of amino acids included in the calculation. The reference protein used was the FAO/WHO EAAS pattern from the joint [20].

2.2.6. Mineral Analysis

Mineral analysis was performed according to Pinto et al. [21]. Sample mineralization was performed by microwave-assisted closed-vessel acid digestion using an MLS-1200 Mega high-performance microwave digestion unit (Milestone, Sorisole, Italy) equipped with an HPR-1000/10 S rotor. Microminerals determination was performed by inductively coupled plasma-mass spectrometry (ICP-MS) using an iCAP™ Q instrument (Thermo Fisher Scientific, Bremen, Germany) and measuring the following elemental isotopes (m/z ratios): ^{7}Li, ^{9}Be, ^{27}Al, ^{51}V, ^{52}Cr, ^{55}Mn, ^{59}Co, ^{60}Ni, ^{65}Cu, ^{66}Zn, ^{75}As, ^{82}Se, ^{85}Rb, ^{88}Sr, ^{114}Cd, ^{133}Cs, ^{137}Ba, ^{205}Tl, ^{208}Pb and ^{209}Bi. The determination of Ca, Mg, Fe, Na and K was performed by flame atomic absorption spectroscopy (FAAS) using a PerkinElmer (Überlingen, Germany) AAnalyst 200 instrument. Limits of detection (LOD) and limits of quantification (LOQ) were estimated from the analysis of 10 digestion blanks. The results are presented in Supplementary Materials (Table S1). For quality control purposes, the certified reference material (CRM) IRMM 807 (rice flour, supplied by EC Institute for Reference Materials and Measurements, Geel, Belgium) and BCR 679 (white cabbage, supplied by EC Institute for Reference Materials and Measurements, Geel, Belgium) were analyzed under the same conditions as the samples. The results obtained were in good agreement with the certified values (recoveries ranging from 95.7% to 108.6%), proving that the method accuracy was adequate.

2.2.7. Estimated Intake of Nutrients and Non-Essential Elements

Evaluation of Nutrient Adequacy

The estimated daily intake (EDI) of nutrients and energy, as % of EFSA dietary reference values (DRVs) [22], was calculated assuming an average daily consumption of 100 g of *nfuma*. DRVs include a set of nutrient reference values: population reference intakes (PRIs), average requirements (ARs), adequate intakes (AIs) and reference intake (RIs). For nutrients that have both PRI and AR, the EDI was calculated based on PRI, since it corresponds to the intake level that meets the needs of all people in a population [22]. Regarding energy, the AR for adults with a physical activity level (PAL) between 1.4 and 2.0 was calculated as the mean value of AR for all age groups between 18 and 79 years. The AI and AR (g/day) for macronutrients were calculated based on the lower and upper limit of AR range for energy.

Estimated Intake of Non-Essential Elements

The estimated intake (EI) was calculated based on the elemental content ($C_{element}$: μg/g), the average per capita consumption of *nfuma* (C_{nfuma}: g) and the adult and toddlers standard human body weight (bw) of 70 and 12 kg [23], respectively, according to the following formula:

$$\text{EI} = \frac{C_{element} \times C_{nfuma}}{\text{bw}} \quad (5)$$

The estimated daily intake (EDI: µg/day/kg bw) of Ni, weekly intake (EWI: µg/week/kg bw) of Al and monthly intake (EMI: µg/month/kg bw) of Cd were calculated assuming a C_{nfuma} of 100 g, 700 g and 3000 g, respectively.

The obtained EI were expressed as % of the toxicological guidance levels for exposure assessment, namely the tolerable daily intake (TDI) [24], the provisional tolerable weekly intake (PTWI) and provisional tolerable monthly intake (PTMI) [25], for Ni, Al, Cd, respectively.

2.2.8. Statistical Analysis

All samples were prepared and analyzed in triplicate. Data were tested for normal distribution of the residuals with Shapiro–Wilk test. The existence of statistically significant differences between means was studied using one-way analysis of variance (ANOVA), when the normal distribution of residuals was confirmed. Welch correction was applied when the homogeneity of variances was not verified. Whenever statistical significance was found, Tukey's or Tamhane's T2 post hoc tests were applied to compare the means, depending, respectively, on equal variance or not. All these statistical analyses were conducted with the XLSTAT for Windows version 2014.5 (Addinsoft, Paris, France) at the 0.05 significance level.

3. Results and Discussion

3.1. Proximate Composition, Energy Value and Sugar Profile

The macronutrient composition of *S. madagascariensis* fruit flour (*nfuma*) is presented in Table 1. The main component was available carbohydrates (49.7–54.9%), followed by fat (26.3–27.8%), total fiber (5.8–10.8%), ash (4.8–6.0%), moisture (~4%) and protein (~3%). *Nfuma* provides an energy value of approximately 475–490 kcal/100 g of flour.

The moisture content of *nfuma* ranged from 4.4 to 4.6%, with no significant differences between the different origins (districts). *Nfuma* showed a low moisture content when compared to cereal flours such as maize and wheat (~13.4) [26]. Compared to commercial fruit flours, it presented a higher content than coconut flour (3.8%), but lower than grape (5%) and other fruit flours (up to 12.2%). The low moisture content of *nfuma*, together with proper packaging, minimizes the risk of microbiological contamination as well as product deterioration during storage, while also improving shelf life.

Protein (3.0–3.5%) was the macronutrient found in the lowest content, similar to flours from other fruits such as green bananas (3.4%) [1] and other common fruits, as described by Carli et al. [27] for 10 different commercial fruit flours (1.59–6.59%). Regarding *Strychnos* spp, Ngadze et al. [9] reported a low protein content for monkey orange, especially for *S. innocua* (0.3–11.5%). [28] also found low protein contents in indigenous fruits (1.3–3.7% dw), reporting 3.3% for *S. spinosa*.

Nfuma has a high-fat content (26.3–27.8%) (Table 1), with the flour originating in Manhiça having the highest value ($p < 0.05$), with no significant differences between flour from other communities. The fat content of *nfuma* is higher than the values reported by Ngadze et al. [9] for all *Strychnos* spp. (0.3–20%), except for *S. spinosa,* where a content of 31.2% dw was reported [29], which was considered an outlier [9]. This flour has an unusual high fat content, even when compared with flours from fat-rich fruits, such as coconut and nuts, because those are by-products of the oil/vegetable milk extraction process [30].

The fixed residue (ash) in *nfuma* ranged from 4.8 to 6.0%. The values obtained are similar to those described for *S. innocua* (4.7%) [31]. Compared to commercial fruit flours studied by Carli et al. [27], passion fruits, papaya and açai presented values (4.9–6.5%) in the same range of *nfuma*.

Table 1. Proximate composition, energy value, sugar and fiber profiles (%) of *S. madagascariensis* fruit flour (*nfuma*) from four districts (Marracuene, Manhiça, Chókwè and Chicualacuala) in southern Mozambique.

	Marracuene	Chókwè	Chicualacuala	Manhiça	*p* Value
Moisture (g)	4.4 ± 0.2	4.6 ± 0.0	4.3 ± 0.1	4.4 ± 0.1	ns
Protein (g)	3.1 ± 0.0 ^{b}	3.2 ± 0.1 ^{b}	3.0 ± 0.0 ^{b}	3.5 ± 0.1 ^{a}	0.003 **
Fat (g)	26.3 ± 0.2 ^{b}	27.0 ± 0.0 ^{b}	26.9 ± 0.1 ^{b}	27.8 ± 0.0 ^{a}	0.016 **
Ash (g)	5.5 ± 0.1 ^{b}	4.8 ± 0.0 ^{c}	6.0 ± 0.1 ^{a}	5.0 ± 0.1 ^{c}	0.020 **
Total Carbohydrates $^{\#}$ (g)	60.7 ± 0.1 ^{a}	60.5 ± 0.1 ^{a,b}	59.8 ± 0.2 ^{a,b}	59.3 ± 0.1 ^{b}	0.026 **
Available Carbohydrates $^{\#}$ (g)	54.9 ± 0.1 ^{a}	49.7 ± 0.1 ^{d}	52.4 ± 0.0 ^{c}	53.1 ± 0.1 ^{b}	0.006 **
Sugars					
Fructose (g)	4.0 ± 0.0	3.5 ± 0.1	3.3 ± 0.2	4.3 ± 0.5	ns
Glucose (g)	4.4 ± 0.2	3.7 ± 0.0	3.7 ± 0.1	5.0 ± 1.0	ns
Sucrose (g)	1.4 ± 0.2	1.8 ± 0.4	1.9 ± 0.4	1.8 ± 0.4	ns
Total dietary fiber (g)	5.8 ± 0.1 ^{c}	10.8 ± 0.0 ^{a}	7.4 ± 0.2 ^{b}	6.2 ± 0.1 ^{c}	0.001 **
Insoluble (g)	5.2 ± 0.1	6.9 ± 0.4	6.0 ± 0.2	5.5 ± 0.2	ns
Soluble (g)	0.6 ± 0.1 ^{c}	3.9 ± 0.4 ^{a}	1.4 ± 0.01 ^{b}	0.6 ± 0.1 ^{c}	0.022 **
Energy (kJ)	2005 ± 8 ^{a,b}	1982 ± 1 ^{b,c}	1995 ± 1 ^{a}	2039 ± 3 ^{a}	0.012 **
(kcal)	480 ± 2 ^{a,b}	476 ± 0.2 ^{b,c}	478 ± 0.2 ^{a}	489 ± 0.7 ^{a}	0.014 **

Data expressed as mean ± standard deviation (n = 3 independent samples × 3 analytical replicates); ns, not significant. Different letters for each district in a row indicate statistically significant differences ($p < 0.05$) between means. p Values from one-way ANOVA. Means were compared by Tukey's since homogeneity of variances was confirmed by Levene's test ($p > 0.05$). ** p Values from one-way Welch ANOVA. Means were compared by Tamhane's T2 test since homogeneity of variances was not confirmed by Levene's test ($p < 0.05$). $^{\#}$ Carbohydrate content was determined by difference [Total carbohydrates = 100 − (moisture + protein + fat + ash); Available carbohydrates = Total carbohydrates − Total dietary fiber].

The most abundant macronutrient of *nfuma* was carbohydrate (~60%). Available carbohydrates varied from 49.7 to 54.9%, with the lowest content found in Chókwè and the highest in Marracuene flour. This value is lower than >80% of total carbohydrates found in other flours, such as cereal and cassava flours [26] or unripe banana and sweet potato flours [32]. In relation to *Strychnos* Spp, lower values of total carbohydrates were described in the Ngadze et al. review [9]; however, Kalenga Saka and Msonthi [29] described a total carbohydrate value of 61% dw in *S. innocua*. Regarding the free sugars, which together represent ~10% of flour mass, fructose and glucose are the ones present in higher levels, ranging between 3.3–4.3% and 3.7–5.0%, respectively, while sucrose represents only 1.4–1.9% of the flour. No significant differences ($p < 0.05$) were observed between sugars profiles of flours from different communities. The total fiber content of *nfuma* varies significantly between communities, with Marracuene flour having the lowest content of total fiber (5.8%) and Chókwè flour the highest one (10.8%). In fact, the highest soluble fiber content in Chókwè (3.9% vs. 0.6–1.4%) is responsible for the higher total fiber content of these flour, since the insoluble fiber value is similar in all flours (5.2–6.9%). Ngadze et al. [9], reported lower values of total carbohydrates for *Strychnos* spp. (*S. innocua* and *S. spinosa*, *S. cocculoides* and *S. pungens*), however Kalenga Saka and Msonth [29] and Lockett et al. [33] described a total carbohydrate value of ~60% for *S. innocua* and *S. spinosa*, respectively. Regarding the sugar profile, the higher ratio of monosaccharides/sucrose agrees with [34], wherein most of the indigenous South African fruits were monosaccharides dominant. This pattern was also observed in several dried fruits [35].

Given the total fiber content (ranging between 6 and 11%), *nfuma* can be considered as a food "high in fiber", according to Regulation (EC) No. 1924/2006 [36], a claim which "may only be made where the product contains at least 6 g of fiber per 100 g". Compared with cassava four (1.6%) and corn flour (2.6%) [18], *nfuma* stands out for its high fiber content. In the study by Carli et al. [27], only coconut (9.4%) and orange (7.6%) flours had similarly high fiber contents. The other fruit flours studied had fiber contents between 2.0–4.9%. Ngadze et al. [9] describe a fiber content of 2.5–22.2% dry weight basis (dw) for other *Strychnos* spp, with the mean fiber content of *S. innocua* (9.4%) the most similar to *nfuma*, the *S. madagascariensis* flour.

3.2. Fatty Acids Composition and Atherogenic and Thrombogenic Indices

Nfuma has a high percentage of monounsaturated fatty acids-MUFA (~17 g/100 g of flour; ~65% of total fatty acids), followed by saturated fatty acids-SFA (~7 g/100 g; ~25% of total fatty acids) and polyunsaturated fatty acids-PUFA (~2.5 g/100 g; ~9% of total fatty acids) (Table 2). In relation to the origin of the flour, significant differences ($p < 0.05$) were observed for the main fatty acids, oleic and palmitic acids, being the flour from Manhiça significantly higher in both fatty acids, which is in line with its higher fat content.

Table 2. Fatty acids composition (mg/100 g of flour) and atherogenic (AI) and thrombogenic (TI) indices of *S. madagascariensis* fruit flour (*nfuma*) from four districts (Marracuene, Manhiça, Chókwè and Chicualacuala) in southern Mozambique.

Fatty Acid	Marracuene	Chókwè	Chicualacuala	Manhiça	*p* Value
C12:0	3.4 ± 0.2	3.5 ± 0.9	3.8 ± 0.4	3.7 ± 0.5	ns
C14:0	34.1 ± 1.9	35.4 ± 1.0	35.3 ± 1.2	36.1 ± 0.5	ns
C16:0	5229.9 ± 118.3 ^{a,b}	5354.5 ± 58.6 ^{a}	5359.1 ± 67.2 ^{a}	5528.3 ± 52.6 ^{b,c}	0.033 *
C16:1-*n*9	421.4 ± 13.5	428.7 ± 3.1	428.5 ± 8.9	439.1 ± 1.8	ns
C18:0	1198.7 ± 13.2	1201.6 ± 23.3	1213.6 ± 15.8	1258.0 ± 24.5	ns
C18:1-*n*9	16,464.1 ± 298.7 ^{a,b}	16,905.2 ± 3.3 ^{c}	16,812.7 ± 147.7 ^{a,c}	17,388.1 ± 25.0 ^{b,c}	<0.001 **
C18:2-*n*6	1791.6 ± 55.4	1832.8 ± 17.6	1839.6 ± 36.2	1884.9 ± 13.0	ns
C18:3-*n*3	457.5 ± 21.1	460.8 ± 9.9	466.0 ± 14.1	472.4 ± 9.6	ns
C20:0	161.0 ± 2.8	165.7 ± 2.5	168.2 ± 5.4	171.8 ± 2.8	ns
C20:1-*n*9	78.1 ± 1.4	82.4 ± 2.7	83.3 ± 3.9	84.8 ± 3.7	ns
SFA	6926 ± 138	7068 ± 67	7084 ± 76	7314 ± 80	-
MUFA	17,013 ± 313	17,469 ± 4	17,390 ± 160	17,965 ± 17	-
PUFA	2304 ± 79	2345 ± 27	2361 ± 51	2408 ± 17	-
AI $^{\#}$	0.28 ± 0.00	0.28 ± 0.00	0.28 ± 0.00	0.28 ± 0.00	-
TI $^{\#}$	0.60 ± 0.00	0.60 ± 0.00	0.60 ± 0.00	0.60 ± 0.00	-

Data expressed as mean ± standard deviation (n = 3 independent samples × 3 analytical replicates); ns, not significant. Different letters for each district in a row show statistically significant differences ($p < 0.05$) between means. * p Values from one-way ANOVA. Means were compared by Tukey's since homogeneity of variances was confirmed by Levene's test ($p > 0.05$). ** p Values from one-way Welch ANOVA. Means were compared by Tamhane's T2 test since homogeneity of variances was not confirmed by Levene's test ($p < 0.05$). $^{\#}$ AI and TI were calculated according to Ulbricht and Southgate [16].

The fatty acid profile of *nfuma* is similar to that of high-fat fruits and their oils, namely olives, avocados and nuts [37,38], but with a slightly higher saturated content. Although *nfuma* has a higher fat content than cereal flour, it presents high amounts of oleic acid (~16 g/100 g of flour), some linoleic (1.8 g/100 g) and alpha-linolenic (0.5 g/100 g) acids and low amounts of SFA, which means it can play a protective role in health. The SFA fraction was mainly palmitic acid (~5 g/100 g), known for its controversial association with detrimental health effects; however, an optimal intake of palmitic acid, in an adequate ratio to unsaturated fatty acids may be crucial to maintain membrane phospholipids balance [39].

Despite the differences in the main fatty acids, regarding the health-related lipid indices of *nfuma*, the atherogenic index (AI) and thrombogenic index (TI) were 0.28 and 0.60, respectively, and did not differ between flour origins. Lower AI and TI values (close to zero) translate into lower atherogenic and thrombogenic potential. Thus, the consumption of *nfuma* can contribute to the prevention of cardiovascular diseases, since this flour, like olive oil (AI = 0.14; TI = 0.32), has AI and TI values below 1 [16]. Higher indices were reported for coconut (AI = 13.63; TI = 6.18) and palm (AI = 2.03; TI = 2.07) lipids, which are mainly characterized by SFA and are associated with cardiovascular diseases [16,40].

3.3. Vitamin E and Carotenoids

Unlike cereal flours, the high-fat content of *nfuma* is also responsible for its high content of liposoluble bioactive compounds, namely vitamin E and β-carotene (provitamin A activity) (Table 3).

Table 3. Vitamin E and β-carotene contents (mg/100 g) of *S. madagascariensis* fruit flour (*nfuma*) from four districts (Marracuene, Manhiça, Chókwè and Chicualacuala) of southern Mozambique.

	Vitamin E	β-Carotene
Marracuene	6.73 ± 0.74	2.56 ± 0.08
Chókwè	7.97 ± 0.72	2.45 ± 0.34
Chicualacuala	6.88 ± 0.13	2.19 ± 0.11
Manhiça	7.44 ± 0.40	2.64 ± 0.13
p value	ns	ns

Data expressed as mean ± standard deviation (n = 3 independent samples × 3 analytical replicates); ns, not significant.

Vitamin E and β-carotene contents ranged between 6.73–7.97 and 2.19–2.64 mg/100 g, respectively. Although no statistically significant differences were observed between communities, Chókwè and Manhiça presented the highest content of vitamin E and β-carotene, respectively. *Nfuma* has a higher β-carotene content than yellow sweet potato flour (0.6 mg/100 g) which is commonly used by local communities in Mozambique to make bread, cakes and porridges [41]. Regarding vitamin E, *nfuma* was shown to have a significant amount, similar to peanuts (9.9 mg/100 g) and some vegetable oils, such as palm oil (9.5 mg/100 g) [26].

3.4. Amino Acids and Protein Nutritional Quality

The amino acid (AA) composition (mg/g protein) of *nfuma* from the different districts is shown in Table 4. Of the 18 amino acids analyzed, 17 were identified in the flour. Arg was the amino acid with the highest amount (148–156 mg/g protein), followed by Asp (106–121 mg/g), Glu (92–103 mg/g) and Ser (93–97 mg/g), Val (73–76 mg/g) and Leu (73–75 mg/g), Thr (58–61 mg/g) and Phe (59–62 mg/g) and Cys (50–56 mg/g), with significant differences between communities ($p < 0.05$) for Arg, Asp and Ser. Protein from Marracuene flour presented the higher Asp and Ser content, and lower of Arg. The lowest AA amounts were found for Met, Trp and Lys (up to 17 mg/g protein), being significantly different between communities, and Ala was not detected. Lys and Met were significantly higher in protein from Chicualacuala flour (16.9 mg/g protein) and Chókwè (15.6 mg/g protein), respectively, and Trp was significantly lower in protein from Marracuene flour (10.3 mg/g protein). Studies on the AA composition of indigenous fruits is scarce in the literature [42], and no information is available for fruits or products of *Strychnos* spp. [9]. Arg, the main AA in *nfuma*, was also found as the main AA of *Dovyalis longispina* and is generally abundant in other indigenous fruits [42]. Glu and Asp, the second and third most abundant AA in *nfuma*, have been described in relatively high amounts in other indigenous fruits [42]. These three AA have been described as major amino acids in nut seeds [38].

Met and Trp were the least abundant AA in *nfuma* (up to 15.6 mg/g protein), which is a common finding for proteins of plant origin [19]. Met was also found to be the least abundant AA by Sibiya et al. [42] in almost all indigenous fruits; however, different results were found for Lys, one of the least abundant AA in *nfuma* (up to 16.9 mg/g protein), but with relatively high or intermediate levels in some indigenous fruits [42]. Lys exhibits significant thermal instability even at low temperatures [19]; therefore, some losses may have occurred during the drying and roasting processes and its concentration may be higher in *S. madagascariensis* fresh fruits. Although in low amounts, *nfuma* presents all nine essential AA, which is in agreement with the findings of Sibiya et al. [42], who found eight essential AA (Trp was not evaluated) in 14 indigenous fruits.

The nutritional quality of the protein of *nfuma*, expressed as essential amino acid scores (EAAS) [20], is presented in Figure 2.

Table 4. Amino acids composition of protein (mg/g protein) from the *S. madagascariensis* fruit flour (*nfuma*) from four districts (Marracuene, Manhiça, Chókwè and Chicualacuala) in southern Mozambique.

Amino Acid	Marracuene	Chókwè	Chicualacuala	Manhiça	*p* Value
Asp	121 ± 1 [b]	106 ± 3 [a]	114 ± 2 [a,b]	116 ± 4 [b]	0.004
Glu	101 ± 5	103 ± 17	92.0 ± 2.6	103 ± 18	ns
Ser	97.2 ± 0.4 [b]	93.2 ± 1.5 [a,b]	96.9 ± 0.5 [a,b]	92.7 ± 2.2 [a]	0.020
His	25.0 ± 2.4	22.6 ± 0.3	24.1 ± 0.6	22.6 ± 0.7	ns
Gly	27.3 ± 0.4	27.3 ± 0.4	27.8 ± 0.2	27.7 ± 0.5	ns
Thr	59.5 ± 0.8	59.1 ± 0.7	61.1 ± 1.7	58.4 ± 1.5	ns
Arg	148 ± 1 [b,c]	153 ± 2 [a,c]	156 ± 1 [a]	153 ± 3 [a,c]	0.041
Ala	nd	nd	nd	nd	
Tyr	42.6 ± 0.3	40.7 ± 0.6	42.7 ± 0.1	40.3 ± 1.5	ns
Val	74.6 ± 0.3	73.1 ± 0.9	75.6 ± 0.3	73.1 ± 2.3	ns
Met	12.0 ± 0.0 [b]	15.6 ± 0.3 [a]	11.5 ± 0.3 [b,c]	10.9 ± 0.2 [c]	<0.001
Phe	59.0 ± 0.2	59.0 ± 0.8	62.4 ± 0.2	58.9 ± 2.4	ns
Ile	43.3 ± 0.6	43.1 ± 1.1	43.8 ± 0.3	42.6 ± 1.1	ns
Leu	72.8 ± 0.3	73.6 ± 1.2	75 ± 0.3	72.7 ± 2.2	ns
Lys	14.9 ± 0.1 [b]	15.6 ± 0.2 [b]	16.9 ± 0.3 [a]	15.4 ± 0.5 [b]	0.002
Pro	41.1 ± 1.5 [a,b]	49 ± 5.2 [b]	30.1 ± 3.1 [a]	43.1 ± 3.6 [b]	0.005
Trp	10.3 ± 0.5 [b]	14.9 ± 0.3 [a]	14.0 ± 0.6 [a]	14.0 ± 0.3 [a]	<0.001
Cys	49.9 ± 1.6	50.4 ± 4.3	56.4 ± 1.3	55.5 ± 1.0	ns
∑AAA [1]	102 ± 0	100 ± 1	105 ± 0	99.2 ± 3.9	-
∑SAA [2]	61.9 ± 0.0	66.8 ± 0.3	68.4 ± 0.3	66.1 ± 0.2	-
∑EAA [3]	464 ± 3	469 ± 6	484 ± 1	464 ± 12	-

Data expressed as mean ± standard deviation (n = 3 independent samples × 3 analytical replicates); nd, below limit of detection (4.06 μM for Ala); ns, not significant. Different letters for each district in a row show statistically significant differences ($p < 0.05$) between means. p Values from one-way ANOVA analysis. Means were compared by Tukey's. since homogeneity of variances was confirmed by Levene's test ($p > 0.05$). [1] Aromatic amino acids: Phe+Tyr; [2] sulfur amino acids: Met+Cys; [3] Sum of essential amino acids Thr+Val+Met(+Cys)+Ile+Leu+Phe (+Tyr)+His+Lys+Trp used for daily requirements and protein value as suggested by FAO/WHO/UNU [20].

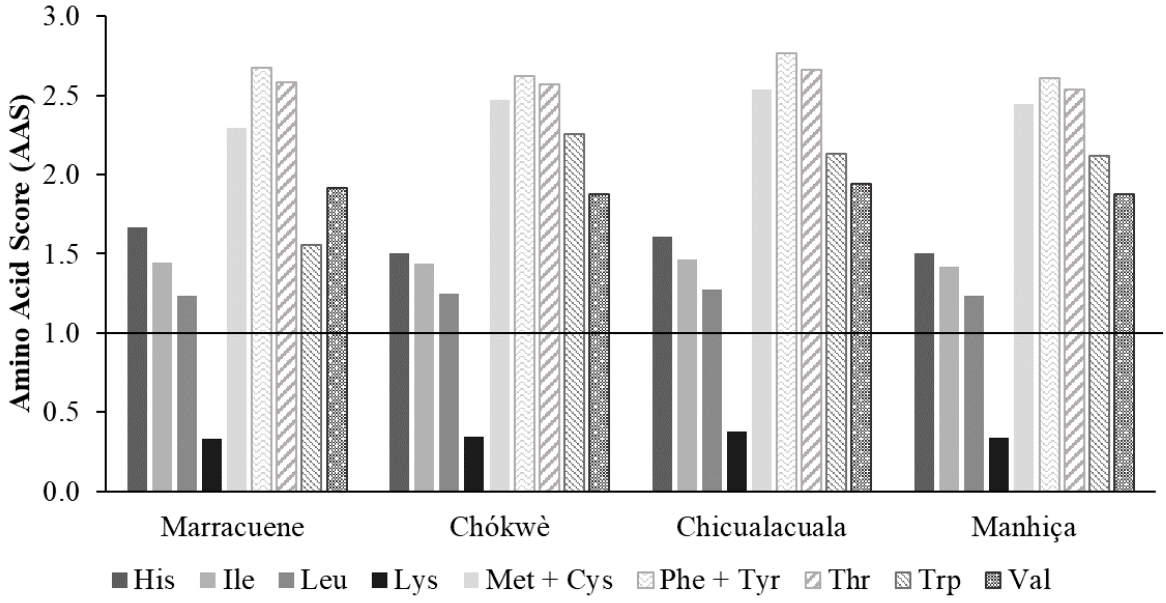

Figure 2. Essential amino acid scores (EAAS) of *S. madagascariensis* fruit flour (*nfuma*) from 4 districts of Mozambique. Adult maintenance patterns are expressed as mg AA/g protein: His. 15; Ile. 30; Leu. 59; Lys. 45; Met + Cys. 27; Phe + Tyr. 38; Thr. 23; Trp. 6.6; Val. 39, according with WHO/FAO/UNU [20].

When all nine individual scores are greater than or equal to 1, the protein is considered complete. Despite having all essential AA, according to the results obtained, the protein of *nfuma* is incomplete, presenting Lys as the limiting AA (AAS < 1), which is in agreement with the results available for the protein of other flours, namely those derived from cereals, e.g., wheat and corn [19], or nut seeds [38]. Although the protein of *nfuma* is incomplete, it can balance other amino acid deficiencies. *Nfuma* is a relatively important source of Met+Cys, Phe+Tyr and Thr (AAS $\geq$ 1). To achieve the recommended daily allowances of all EAA, local communities should combine *nfuma* with other protein sources, namely legumes such as beans, as they are a rich source of Lys and a poor source of Met [19]. Beans are mainly grown in rural areas of Mozambique and can help to alleviate malnutrition [43]. The EAA with the highest ratio to daily requirements was Phe+Tyr. These AA are precursors of the physiologically active molecules catecholamines, which act as both neurotransmitters and hormones [44]. Although the three most abundant AA in *nfuma* are considered non-essential, it has been shown that Arg, Asp and Glu (Table 4) can act as regulators of key metabolic pathways, leading to a new concept of functional AA [45].

3.5. Mineral Elements

Table 5 presents the results of mineral content in *nfuma* from different origins (districts). Of the 25 elements analyzed, the content of eight of them (Li, Be, As, Se, Cs, Pb, Tl and Bi) was below the limits of detection. The most abundant macromineral was K (ranging from approximately 1200 to 1700 mg/100 g) and the less abundant was Na (4.0–6.6 mg/100 g).

Table 5. Mineral composition of the *S. madagascariensis* fruit flour (*nfuma*) from four districts (Marracuene, Manhiça, Chókwè and Chicualacuala) in southern Mozambique.

Element	Marracuene	Chókwè	Chicualacuala	Manhiça	*p* Value
Essential macrominerals (mg/100 g)					
Ca	24.7 ± 0.2	31.3 ± 6.3	26.2 ± 0.0	29.9 ± 2.5	ns
Mg	85.6 ± 2.8 ^{a}	80.8 ± 3.4 ^{a,b}	75.3 ± 4.7 ^{a,b}	69.4 ± 4.3 ^{b}	0.025 *
K	1654 ± 102 ^{a}	1399 ± 71 ^{b}	1303 ± 15 ^{b}	1204 ± 80 ^{b}	0.002 *
Na	4.9 ± 0.2 ^{b}	4.0 ± 0.1 ^{b}	6.6 ± 0.1 ^{a}	4.9 ± 0.8 ^{b}	0.002 *
Essential trace elements (µg/100 g)					
Fe	1683 ± 71	1620 ± 56	1476 ± 73	1706 ± 122	ns
Zn	261.4 ± 11.2	216.5 ± 12.5	205 ± 13.4	228.6 ± 20.8	ns
Mn	4017 ± 141	3885 ± 71	3874 ± 21	4098 ± 181	ns
Cu	215.2 ± 1.0 ^{a}	200.3 ± 5.9 ^{a,b}	193.9 ± 7.7 ^{b}	208.5 ± 5.1 ^{a,b}	0.021 *
Cr	58.1 ± 1.4	55.2 ± 2.7	57.5 ± 1.9	58.1 ± 0.5	ns
Co	7.6 ± 0.3	7.3 ± 0.2	6.8 ± 0.3	7.6 ± 0.0	ns
Non-essential trace elements (µg/100 g)					
Al	2631 ± 210 ^{a}	3042 ± 221 ^{a,b}	2995 ± 73 ^{a}	3331 ± 26 ^{b}	0.026 **
Rb	1100 ± 19 ^{a}	980 ± 29 ^{b}	968 ± 15 ^{b}	968 ± 31 ^{b}	0.002 *
Ni	463.8 ± 3.8	482.6 ± 20.5	458.8 ± 12.6	478.0 ± 20.0	ns
Sr	258.2 ± 2.6	240.9 ± 5.5	250.2 ± 14.4	256.2 ± 4.8	ns
Ba	222.3 ± 5.1 ^{b}	252.2 ± 8.6 ^{a}	249.7 ± 9.5 ^{a}	257.0 ± 4.6 ^{a}	0.006 *
V	17.9 ± 0.4 ^{a}	17.6 ± 0.5 ^{a}	17 ± 0.6 ^{a}	21.9 ^{b} ± 0.3	0.002 *
Cd	2.4 ± 0.0	2.1 ± 0.2	2.3 ± 0.1	2.1 ± 0.1	ns

Data expressed as mean ± standard deviation (n = 3 independent samples × 3 analytical replicates); ns, not significant. Different letters for each district in a column show statistically significant differences ($p < 0.05$) between means. * p Values from one-way ANOVA. Means were compared by Tukey's since homogeneity of variances was confirmed by Levene's test ($p > 0.05$). ** p Values from one-way Welch ANOVA. Means were compared by Tamhane's T2 test since homogeneity of variances was not confirmed by Levene's test ($p < 0.05$).

This trend was also observed in other fruit flours [1,27], dried fruits [46] and indigenous fruits [28,47]. Brito et al. [1] found similar values for green banana flour, with mean value of 1100, 88, and 45 mg/100 g for K, Mg and Ca, respectively. Commercial fruit

flours (up to 952 mg/100 g) [27] and dried fruit products (up to 1162 mg/100 g), have been described as essential sources of K [46]; however, *nfuma* presents even higher values. Among the 14 wild fruits native to southern African studied by Sibiya et al. [47], higher K levels were found in *Carissa macrocarpa* and *Syzygium cordatum* (1312.3 and 1427.1 mg/100 g dw, respectively). Regarding *Strychnos* spp, our results for *S. madagascariensis* flour are in the range of those observed by Kalenga Saka and Msonthi [29] and Amarteifio and Mosase [28] for *S. spinosa* fruit (1968 and 1370 mg/100 g dw, respectively) for K, and higher for Mg (43 and 49 mg/100 g dw, respectively).

For essential macrominerals, statistically significant differences were observed between the content of K, Mg and Na in the flours of the different communities (Marracuene, Chókwè, Chicualacuala and Manhiça). A high K and Mg content was observed for Marracuene flour (1654 ± 102 and 85.6 ± 2.8 mg/100 g, respectively) and a high Na content was observed for Chicualacuala flour (6.6 ± 0.1 mg/100 g).

Regarding essential trace elements, the following trend was observed: Mn (~4000 μg/100 g) > Fe > Zn > Cu > Cr > Co (6.8–7.6 μg/100 g). Significant differences ($p < 0.05$) were observed only for Cu (higher Cu content in Marracuene flour compared to Chicualacuala flour). *Nfuma* can be considered an interesting source of Mn when compared to dried fruits (~300 μg/100 g: apricot, dates, peach, poir, runes, raisins) [46]. Of the commercial fruit flours studied by Carli et al. [27], only coconut showed a Mn concentration similar to *nfuma*. Hassan et al. [48] found a Mn content of 2500 μg/100 g dw for *S. innocua* fruit. For Fe (~1600 μg/100 g) and Zn (~250 μg/100 g) in *nfuma*, lower levels were found when compared to cashew flours (~5000 and 4000 μg/100 g, respectively) [49]. Carli et al. (2017) reported higher Fe values in 8 out 10 fruit flours (~3000–11,000 μg/100 g: plum, coconut, orange, papaya, apple, passion fruit, green banana flours, in ascending order) and all flours had higher Zn values (500 to 4000 μg/100 g) than *nfuma*. Amarteifio and Mosase [28] reported a similar Zn content (220 μg/100 g dw) for *S. spinosa* fruit, and a lower value for Fe (~110 μg/100 g dw), proposing the supplementation of these fruits to meet Fe requirements. Interestingly, several studies report the ability of *Strychnos* spp. to improve nutrition based on their high Fe and Zn contents [9,10,50], despite the wide variation between and within the *Strychnos* spp. (other than *S. madagascariensis*), as reviewed by Ngadze et al. [9]. The Cu content (200 μg/100 g) of *nfuma* was in agreement with the data for some fruit flours, as reported by Carli et al. [27] (~170–210 μg/100 g for açai, orange and lemon flours) and Brito et al. [1] (up to 300 μg/100 g for apple and green banana). Regarding Cr, *nfuma* has contents (~58 μg/100 g) higher than those reported by Brito et al. [1] for apple and green banana flours (up to 20 μg/100 g). The minor essential element found in *nfuma* was Co (~7 μg/100 g), a constituent of vitamin B12. *Nfuma* presents higher Co levels than grain flours (up to 1.2 μg/100 g) [51] and within the range of dried sweet cherries (~0.6 to 14 μg/100 g; mean value of 3 μg/100 g dw) [52]. For *S. innocua*, Hassan et al. [48] reported quite different Co levels compared to *nfuma*, reaching 1200 μg/100 g dw.

Some non-essential elements were also quantified in *nfuma*. Among those, Al was the most abundant (~2600–3300 μg/100 g) and Cd the least (~2.1–2.4 μg/100 g). Statistically significant ($p < 0.05$) were observed for Al, Rb and Ba, with a higher Rb content and a lower Ba content in the Marracuene flour. For Al, a higher content was observed for Manhiça flour compared to Marracuene flour (3331 ± 26 vs. 2631 ± 210 μg/100 g). Aluminum content can vary significantly in food, depending on the food composition itself as well as on "external" factors (e.g., soil contamination, culinary practices). Our results are in close agreement with Brito et al. (2017), who studied two fruit flours (apple and green banana) and found Al levels ranging from 190 to 4900 μg/100 g.

3.6. Nutrient Adequacy of Nfuma

The estimated daily intake of nutrients and energy, expressed as % of DRV, was calculated assuming an average per capita consumption of *nfuma* of 100 g per day and is presented in Table 6. For macronutrients, except protein and total dietary fiber, the EDI was calculated based on the energy value. Thus, macronutrients DRVs depend on each

individual's energy needs. Overall, consumption of 100 g of *nfuma* contributes to 15–27% of daily energy needs, depending on the physical activity level. EDI values show that *nfuma* is an important source of fiber and lipids, namely alpha-linolenic acid, contributing to 30%, 22–69% and 27–48% of DRVs, respectively. Regarding liposoluble vitamins, *nfuma* provides 55–63% and 56–66% of vitamins A and E, respectively. In Mozambique, 69% of children under 5 years of age are deficient in vitamin A (Amaro, 2019; World Health, 2006). Considering a daily consumption of 50 g of *nfuma*, children aged 1–3 and 4–6 years can obtain 82 and 68% (Table 7), respectively, of vitamin A EDIs [22]. Thus, the consumption of *nfuma* by children in Mozambique may alleviate vitamin A deficiency due to its high β-carotene content. As mentioned above, flour is used during times of food shortage as a supplement to staple foods, such as maize and cassava flour. Maize flour is often boiled in water to make a maize-meal porridge that is consumed for breakfast by communities in sub-Saharan Africa [9]. According to the FAO Food Balance Sheets, Mozambican communities consume 192 g of maize and 285 g of cassava per day [53]. Maize and cassava flour have a higher carbohydrate content (75% and 85%, respectively), while *nfuma* has more fiber, fat and liposoluble vitamins [26]. On the other hand, *nfuma* has a low protein content, providing only 6% (male) and 7% (female) of DRVs. Therefore, as noted above, *nfuma* must be combined with other protein sources to achieve DRVs.

Table 6. Estimated daily intake (EDI), expressed as % of the dietary reference value (DRV) [22], of energy, macronutrients, vitamins and essential elements for adults considering an average per capita *nfuma* consumption of 100 g/day.

	DRV (AI [a]/AR [b]/PRI [c]/RI [d]/SAI [e])		EDI (% DRV)	
	Male	**Female**	**Male**	**Female**
Energy (MJ/day)				
Energy	9.1–13.0 [b,*]	7.4–10.5 [b,*]	15–22	19–27
Macronutrients (g/day)				
Protein	53.1 [c,#]	45.6 [c,#]	6.0	7.0
Fat	48.3–120.8 [d]	38.9–97.3 [d]	22–56	28–69
Alpha-linolenic acid	1.2–1.7 [a]	1.0–1.3 [a]	27–38	33–48
Linoleic acid	9.7–13.8 [a]	7.8–11.1 [a]	13–19	17–24
Carbohydrates	244.5–465.8 [d]	197.1–375.5 [d]	11–22	14–27
Total Dietary Fiber	25 [a]		30	
Vitamins				
Vitamin A (μg RE/day)	750 [c]	650 [c,§]	55	63
Vitamin E (mg/day)	13 [a]	11 [a]	56	66
Minerals (mg/day)				
Ca	950 [c]		3	
Mg	350 [a]	300 [a]	22	26
K	3500 [a]		40	
Na	2000 [e]		0.3	
Fe	11 [c]	16 [c,§]	15	10
Zn	9.4–16.3 [c,†]	7.5–12.7 [c,†]	1.4–2.4	1.8–3.0
Mn	3 [a]		132	
Cu	1.6 [a]	1.3 [a]	12.8	15.7

* Average requirement for adults (18–79 years) with a physical activity level (PAL) between 1.4 and 2.0. # Population reference intake for men and women with a reference body weight of 64 and 55 kg, respectively, based on IMC of 22 kg/m^2. § Population reference intake for premenopausal women. † Population reference intake for adults (≥18 years) with a phytate intake level between 300 and 1200 mg/day. DRV: dietary reference value; [a–e] reference value applied: AI [a]: adequate intake; AR [b]: average requirement; PRI [c]: population reference intake; RI [d]: reference intake; SAI [e]: safe and adequate intake. EDI of vitamin A was calculated based on the conversion of β-carotene content (expressed as mg/100 g) to retinol equivalent (1 μg RE = 6 μg of β-carotene).

Table 7. Estimated daily intake (EDI), expressed as % of the dietary reference value (DRV) [22], of vitamin A and Fe for children (1–6 years) considering an average per capita *nfuma* consumption of 100 g/day.

	DRV (PRI)		EDI (% DRV)	
	1–3 Years	**4–6 Years**	**1–3 Years**	**4–6 Years**
Vitamin A (μg RE/day)	250	300	82	68
Fe (mg/day)	7		12	

DRV: dietary reference value; population reference intake. EDI of vitamin A was calculated based on the conversion of β-carotene content (expressed as mg/100 g) to retinol equivalent (1 μg RE = 6 μg of β-carotene).

Considering the EDI of essential elements (expressed as % of DRV), *nfuma* contributes significantly to the daily intake of Mg and K, representing 22–26% and 40% of DRVs, respectively. In addition, daily consumption of 100 g of *nfuma* is sufficient to meet Mn requirements, since it provides more than 100% of Mn DRV. *Nfuma* has a higher content of specific minerals, especially K and Mg, compared to common staple flours in Mozambique, such as maize (120 mg/100 g of K and 46 mg/100 g of Mg) and cassava flours (20 mg/100 of K and 2 mg/100 g Mg) [26]. On the other hand, 100 g of *nfuma* provides only 10% (female) to 15% (male) of Fe DRV, although it contains twice the Fe content of maize flour (800 μg/100 g). For children (1–6 years), 50 g of *nfuma* provides 12% of Fe DRV (Table 7).

Consequently, the combination of maize flour with *nfuma* in porridges, together with consumption of *nfuma* as a daytime snack, as reported by people from Mozambican communities, may increase Fe intake, which is of great importance given the high prevalence (64%) of anemia in children in Mozambique [54]. However, it is still necessary to study the bioaccessibility and bioavailability of *nfuma* minerals to define its real potential in human nutrition.

3.7. Exposure Assessment to Non-Essential Trace Elements

Considering the non-essential trace elements for which a tolerable intake is established, the amount of Ni, Al and Cd in *nfuma* contributes to 52%, 15% and 9% of the corresponding TDI, PTWI and PTMI, respectively, for adults (Table 8). Thus, the consumption of *nfuma* (100 g) alone is not likely to be considered a relevant source of Al and Cd. The same is not true for Ni, since the average content found contributed to ~50% of the TDI established by the European Food Safety Authority (13 μg/kg bw).

Table 8. Estimated daily (EDI), weekly (EWI) and monthly (EMI) intake, expressed as % of toxicological guidance values of Ni, Al and Cd, considering the consumption of 100 g/day (adults) or 50 g/day (toddlers) of *nfuma*.

Element	**Reference Value**	**Estimated Intake of Non-Essential Elements**	
Ni	TDI (μg/day/kg bw)	EDI (% TDI)	
		Toddlers	Adult
	13	151	52
Al	PTWI (μg/week/kg bw)	EWI (% PTWI)	
		Toddlers	Adult
	2000	44	15
Cd	PTMI (μg/month/kg bw)	EMI (% PTMI)	
		Toddlers	Adult
	25	11	4

TDI: tolerable daily intake (EFSA 2019); PTWI: provisional tolerable weekly intake; PTMI: provisional tolerable monthly intake (JEFCA 2021).

When looking at the exposure of toddlers, the consumption of 50 g of *nfuma* can contribute to 151%, 44% and 11% of the TDI, PTWI and PTMI of Ni, Al and Cd, respectively. These results indicate that young age groups can be at high risk of health complications due to Ni exposure. Since the fruits used to prepare the flour had low levels of Al and

Ni (data not shown), the selection of appropriate materials and the use of good practices in the preparation of *nfuma* should be evaluated in order to mitigate the presence of these non-essential elements, while maintaining the nutritional advantages of the food discussed above.

4. Conclusions

This research aimed to determine the nutritional composition of *nfuma*, a flour from *S. madagascariensis* pulp fruit, prepared by local communities in Mozambique and evaluate its adequacy in terms of nutrient recommendations. This fruit flour stands out for its high fat content, mainly composed by MUFA, delivering vitamin E and carotenes, together with naturally occurring sugars and high fiber content. *Nfuma* is also a good source of Mn and K and, despite being a poor source of Fe, *nfuma* contains twice the Fe content of maize flour. However, its Ni content should be addressed with caution and mitigation strategies are required in order to guarantee its safety.

Although *nfuma* bioaccessibility evaluation is still needed, its consumption seems to be a promising food-based strategy to alleviate the high prevalence of anemia and vitamin A deficiency in children of Mozambique. Its local use in the "enrichment" of maize-based porridges or as ingredient for pastry and snacks for the development of healthier new food products deserves to be technologically approached for wider valorization.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/foods11040616/s1, Table S1: Limits of detection (LOD) for the elements analyzed by ICP-MS.

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Identifying the Food Sources of Selected Minerals for the Adult European Population among Rice and Rice Products

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Abstract: The problem of dietary deficiency of several essential elements among different stages of life is still observed. The consumption of whole grains (among others unprocessed rice) is recommended as a part of a healthy diet. This research aimed to determine the content of selected macro- and microelements in rice and rice products to verify whether the tested products could be regarded as a source of selected minerals in the diet of the adult European population. Methods: A total of 99 samples from 12 groups of rice products (basmati, black, brown, parboiled, red, wild, white rice and expanded rice, rice flakes, flour, pasta, and waffles) were obtained. The atomic absorption spectrometry method (AAS) was used to determine the content of Ca, Cu, Fe, Mg, Mn, Se and Zn in the study material. Results: The average measured contents of Ca, Cu, Fe, Mg, Mn, Se and Zn were as follows: 226.3 ± 160.6 mg/kg, 3.6 ± 2.8 mg/kg, 9.4 ± 7.0 mg/kg, 618.0 ± 498.4 mg/kg, 16.7 ± 10.0 mg/kg, 242.9 ± 140.4 µg/kg and 19.5 ± 15.0 mg/kg, respectively. Statistical analyses confirmed the differences in the levels of the studied elements between the subgroups of processed and unprocessed products. Considering the tolerable upper intake level of studied elements, the tested products could be regarded as safe to consume. Conclusion: All tested products can be recommended as a source of Cu, Mn, and Se, while a majority of studied products can be considered a source of Mg and Zn in the diet of the adult European population.

Keywords: trace elements; nutrients; rice; rice products; dietary sources; human health

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1. Introduction

The consumption of whole grains (unprocessed maize, oats, wheat and unpolished rice) is recommended as a part of a healthy diet. According to the Food and Agriculture Organization (FAO, Geneva, Switzerland) statistics, in 2020, global cereal production reached 2.7 billion tonnes for which rice was the second-largest crop after wheat (764.9 and 509.1 mln of tonnes, respectively). The predominant part of the rice available on the world market is produced in Asian countries (>90%); only 0.6% is cultivated in European countries. The world's three top producers of rice include China followed by India and Indonesia. The highest average rice consumption between 2016 and 2018 in Asian and Pacific countries was estimated at 77.6 kg per capita per year, while the lowest was in the European countries with 5.8 kg per capita per year [1].

Rice is one of the most important crops considering the total production as well as consumption worldwide. Two rice species—*Oryza sativa* S. (Asian rice) and *Oryza glaberrima* L. (African rice)—are cultivated. However, *O. sativa* is the predominant species, grown across the world; in turn, *O. glaberrima* is cultivated mainly in Africa and differs only slightly in its morphological aspects [2]. In the structure of paddy rice grains, two main parts can be specified: the hull and the caryopsis. The hull located outside the grain is rich in minerals and constitutes about 20% of the whole grain. The caryopsis is under the hull

and includes the bran layer, endosperm and germ. The germ is the most nutritious part, rich in protein, fatty acids and mineral compounds [3]. Depending on the processes carried out on the grain, the rice can be classified as paddy (kernels are present inside the hull), brown (when the caryopsis is removed in the hulling process), white or milled (the milling process removes the bran layer and the germ from the brown rice) or parboiled (soaked in hot water then steamed before drying) [4]. White rice has a lower content of protein (especially a reduced lysine amount), minerals and fiber in comparison to brown rice. The parboiling process reduces the glycemic index and additionally contributes to the transfer of some minerals into the grain, which has a positive effect on its nutritional value [4].

It is observed that high consumption of white rice is related to increased risk of impaired glucose homeostasis and the occurrence of type 2 diabetes mellitus (T2DM). On the other hand, black rice intake is related to a lower postprandial blood glucose response (the glycemic index) and consequently, reduced risk of metabolic disorders, such as obesity, hyperglycemia, dyslipidemia, T2DM and hypertension. The anti-cancer activity of black rice bioactive compounds has also been demonstrated [4].

Well-balanced nutrition should provide not only adequate amounts of essential nutrients, such as proteins, carbohydrates and fats as well as vitamins and minerals. According to the available literature, the problem of unbalanced diets is still observed. The inadequate intake of macroelements such as calcium (Ca) or magnesium (Mg) was reported [5]. On the other hand, the over intake of copper (Cu) and manganese (Mn) were determined [6].

Ca plays a crucial role in the mineralization of bones and teeth, takes part in neuromuscular conduction and is required in maintaining the balance of body fluids within cells. Inadequate dietary intake of Ca may result in increased bone mass loss and osteoporotic fractures in old age [7].

Cu is essential for the functioning of several physiological pathways due to being a cofactor or structural component of many enzymes (e.g., cytochrome c oxidase, ceruloplasmin). Cu is important in the neuromodulation process, in response to low blood pressure as well as in angiogenesis. In the case of insufficient intake of Cu with diet, hematological manifestations (such as anemia), bone abnormalities or neurological symptoms may occur [8].

Iron (Fe) is one of the key elements in hematopoiesis. Furthermore, Fe is important for oxygen transport in the human body and is an important element in several metabolic processes, such as the synthesis of deoxyribonucleic acid (DNA) and the transport of electrons. Fe deficiency may lead to the development of anemia, which adversely influences cognitive functions, learning ability and immunity responses. Anemia is particularly dangerous in pregnancy because it is related to several negative outcomes, such as low birth weight or maternal and perinatal mortality [9].

Mg is involved in more than 300 biochemical reactions in the body as a cofactor of enzymes. It takes part, among others, in the production of energy, neuromuscular conduction, bone mineralization, DNA and RNA synthesis. Inadequate Mg intake increases the risk of the development of numerous chronic diseases, such as insulin resistance, T2DM, hypertension, Alzheimer's disease or osteoporosis [10].

Mn is the next element, which participates in a variety of metabolic reactions in an organism. Mn is essential for the proper functioning of the immune, nervous and reproductive systems. Moreover, Mn plays a role in antioxidant responses, energy metabolism and inactivation of metalloenzymes and enzymes crucial for the synthesis of neurotransmitters. Insufficient Mn intake may be associated with impaired metabolism of proteins, fats and carbohydrates, growth disturbances and fertility problems. Excessive Mn intake may lead to the development of a neurodegenerative disorder, called manganism, which causes dopaminergic neuronal death [11].

Selenium (Se) is a trace element characterized by a narrow range between physiological status, deficiency and excessive (toxic) concentration. At the same time, Se is an important element in many physiological processes directly or indirectly. It constitutes a part of antioxidant enzymes (e.g., glutathione peroxidases), supports the response of the

immune system and is required for maintaining reproductive health. Se is essential for proper thyroid functioning by being a part of iodotyrosine deiodinase, which catalyzes the deiodonization of thyroxine (T4) to triiodothyronine (T3). Considering the adverse health effects of inadequate Se intake, disorders of the heart muscle and joints are mainly observed. Increased risk of infertility and prostate cancer in men and neurological impairments are also possible [12].

Zinc (Zn) plays numerous roles in the human body, which could be classified as regulatory, catalytic and structural functions. Among the most important Zn functions, the effect on wound healing, supporting the immune and reproductive systems, regulation of blood pressure and heart rate as well as proper insulin secretion are described. Zn deficiency may impair the functioning of the immune, reproductive, nervous and gastrointestinal systems [13].

Because of the lack of favorable conditions to grow, rice species are not cultivated in Poland. Rice grains available on the Polish market are imported from different world regions, mainly from Asia, Southern Europe and Southern Africa. In this study, we were interested in the assessment of the nutritionally important components of which include macro- and microelements of different rice grains and rice products. To the best of our knowledge, such a broad group of different types of rice products has not yet been studied so far. We collected a broad range of diverse products, including seven rice subgroups and five subgroups of rice products.

In our research, we aimed to determine the content of selected macro- (Ca and Mg) and microelements (Cu, Fe, Mn, Se, and Zn) in rice and rice products, which has not been investigated yet. Moreover, we estimated whether the tested products can be regarded as a food source of the mentioned elements in the diet of the adult European population.

2. Materials and Methods

2.1. Sample Collection

The samples, representative of the overall Polish market, were obtained from local markets in north-eastern Poland. A total of 99 products were acquired between March and May 2020. We collected twelve subgroups of products (at minimum five samples each), among which seven were different types of rice: basmati (n = 10), black (n = 6), brown (n = 10), parboiled (n = 10), red (n = 5), wild (n = 5), white (n = 11). Moreover, we also obtained five different types of rice products: expanded rice (n = 8), rice flakes (n = 12), flour (n = 6), pasta (n = 7), and waffles (n = 9). The samples were not duplicated; each one of the products in the subgroups (e.g., among flakes) was purchased from a different producer. Considering the country of origin, the lack of this information in 19 products was observed. Most of the samples collected were imported from Asian countries (n = 53), while 27 were imported from Europe.

2.2. Sample Digestion

The preparation of samples for the mineralization process included homogenization in the stainless-steel mill. The samples were not treated thermally prior to the analysis. An appropriate amount (0.2–0.3 g) was weighted and transferred into mineralization vessels, then 4 mL spectrally pure concentrated (69%) nitric (V) acid was added (Tracepur, Merck, Darmstadt, Germany). A close-loop system was used to perform microwave digestion (Berghof, Speedwave, Eningen, Germany).

2.3. Analysis of Studied Elements Contents

The content of selected elements in the digested rice samples was determined by atomic absorption spectrometry (AAS), using the Z-2000 instrument (Hitachi, Tokyo, Japan). Before the analysis, the majority of the mineralized samples were diluted 20 times for Ca and Cu, 5 for Fe, 50 for Mg, 100 for Mn, 2 for Se and 10 for Zn. In the case of Cu, Mn, and Se, the flameless AAS technique with electrothermal atomization in a graphite cuvette was applied. To determine the Se content, the palladium–magnesium matrix modifier

(Merck, Darmstadt, Germany) was added (Pd concentration: 1500 mg/L; Mg concentration: 900 mg/L), while for the Mn measurement, magnesium nitrate ($Mg(NO_3)_2$ concentration: 100 mg/L, Sigma-Aldrich, Merck, Darmstadt, Germany) as a modifier was used. The flame AAS technique in the acetylene–air flame with Zeeman background correction for the determination of Ca, Fe, Mg, Zn was used. Moreover, in the case of Ca and Mg, 1% lanthanum chloride ($LaCl_3$, Sigma-Aldrich, Merck, Darmstadt, Germany) was adopted as a masking agent. The analytical conditions of the process of the flameless and flame techniques are presented in Table 1.

Table 1. The analytical conditions of flameless AAS technique (Cu, Mn and Se) and flame AAS technique (Ca, Fe, Mg and Zn) in determining the content of elements in rice samples.

	Element						
Parameter	**Cu**	**Mn**	**Se**	**Ca**	**Fe**	**Mg**	**Zn**
Wavelength (nm)	324.8	279.5	196.0	422.7	248.3	285.2	213.9
Lamp current (mA)	7.5	7.5	14.5	7.5	12.5	7.5	6.5
Drying (°C)	80/140	80/140	70/100	-	-	-	-
Ashing (°C)	600/600	750/750	600/600	-	-	-	-
Atomization (°C)	2400/2400	2300/2300	2700/2700	-	-	-	-
Cuvette cleaning (°C)	2500/2500	2500/2500	2800/2800	-	-	-	-

2.4. Method Validation

The certified reference materials (CRMs) were used to control the quality of performed analyses (Table 2). In the case of Ca, Cu, Fe, Mg, Mn, and Zn, corn flour (INCT–CF-3) was applied, while for Se, mushroom powder (CS–M-3) was used (both standards produced by the Institute of Nuclear Chemistry and Technology, Warsaw, Poland). The CRMs were analyzed before and for every ten determinations.

Table 2. The results obtained in the method validation.

Element	Detection Limit for Method *	Detection Limit for Samples [1]	Recovery for CRM	Precision (%)
Ca	0.10 mg/L	9.26 mg/kg	98.4	3.6
Cu	0.65 µg/L	0.24 mg/kg	99.2	2.1
Fe	0.11 mg/L	1.29 mg/kg	98.7	2.5
Mg	0.009 mg/L	3.33 mg/kg	98.8	2.7
Mn	0.14 µg/L	0.13 mg/kg	101.2	2.4
Se	1.55 µg/L	57 µg/kg	97.5	4.7
Zn	0.015 mg/L	1.39 mg/kg	100.9	1.8

* Estimated as a characteristic concentration in standard solution for 0.0044 absorbance. [1] Detection limit considering amount weighted and diluted for samples.

2.5. Assessment Whether Tested Products Could Be Regarded as a Source of Studied Nutrients

We assessed whether the tested groups of products contained in 100 g, at minimum, 15% of the reference value intake (RVI) established by the European Parliament and of the Council. If this requirement was met, the product could be regarded as a source of the studied elements for the adult European population [14].

2.6. Estimation of the Risk of Adverse Health Effects

Based on the results obtained in this research and the tolerable upper intake level (TUIL) determined by the National Institutes of Health in the United States of America, the risk of adverse health effects resulting from daily consumption of the studied nutrients was evaluated. The limits considered the highest daily nutrient intake, which should pose no adverse health effects to almost all individuals in the general population. The following levels for adults over 19 years of age were established: Cu, 10 mg/day; Fe, 45 mg/day;

Mn, 11 mg/day; Se, 400 μg/day; and Zn, 40 mg/day. In the case of Ca, two age groups were specified: 2500 mg/day (for adults aged 19–50 years) and 2000 mg/day (for adults over 50 years). For Mg, the TUIL was given for pharmacological agents use and did not include intake from food and water; therefore, it was not analyzed in our research [15]. The maximum amounts of safe intake levels of studied products were calculated.

2.7. Statistical Analyses

Statistica software (Tibco, Palo-Alto, CA, U.S.A.) was used to analyze the data. The normality distribution of the data was checked by the Shapiro–Wilk test. The results were shown as mean (X) with standard deviation (SD), minimum (Min), maximum (Max) as well as the median and interquartile range (lower quartile Q_1, upper quartile Q_3). Kruskall–Wallis Analysis of Variance (ANOVA) with post-hoc analysis to compare the content of the studied elements between the subgroups of products was performed. Significant differences were assumed at p-values < 0.001, < 0.01 and < 0.05.

3. Results

The results obtained in our analysis are presented in Tables 3 and 4.

None of the subgroups tested can be considered a source of Ca and Fe in the diet of the adult European population (the RVI was lower than 15%). Contrastingly, every studied subgroup could be taken into account as a source of Cu, Mn, and Se. In the case of Mg, six subgroups (basmati, expanded, parboiled, white rice, flakes and pasta) and three of Zn (basmati, parboiled rice and pasta) did not meet the requirements; therefore, they cannot be regarded as a source of these nutrients in the diet (Tables 3 and 4).

The TUIL for Ca in every subgroup was higher than 7000 g/day, while that for Fe was higher than 2570 g/day. TUIL for Cu ranged from 811 g/day for wild rice to 4579 g/day for white rice; for Mn, it was lowest for red rice (298 g/day) and highest for parboiled rice (5300 g/day). In the case of Se, the lowest TUIL was calculated for parboiled rice (1297 g/day) and at the same time, the highest (2321 g/day) for wild rice. The TUIL for Zn ranged from 672 g/day for wild rice to 7265 g/day for parboiled rice.

The statistical analysis confirmed the differences in the content of studied elements between the unprocessed (e.g., black or red rice) and processed (e.g., flakes or pasta) subgroups of products. The Kruskall–Wallis Analysis of Variance (ANOVA) test with post-hoc analysis was used. Only in the case of Cu, no differences were found between the studied products. The differences between the contents of the studied elements in the studied product groups are shown in Table 5. The p-values (p < 0.05, 0.01, 0.001) were placed in superscript.

Table 3. The content of studied elements (Ca, Cu, Fe, Mg) measured in rice and rice products.

The Type of the Rice and Rice Product	*n*	Ca (mg/kg)			Cu (mg/kg)			Fe (mg/kg)			Mg (mg/kg)		
		X ± SD (Min–Max)	Me (Q_1–Q_3)	% of RVI (800 mg)	X ± SD (Min–Max)	Me (Q_1–Q_3)	% of RVI (1 mg)	X ± SD (Min–Max)	Me (Q_1–Q_3)	% of RVI (14 mg)	X ± SD (Min–Max)	Me (Q_1–Q_3)	% of RVI (375 mg)
Basmati	10	193.5 ± 61.9 (133.3–343.9)	181.9 (151.8–208.3)	2	2.4 ± 0.4 (1.9–3.1)	2.2 (2.0–2.6)	24	6.5 ± 7.9 (2.4–28.9)	4.0 (3.4–5.1)	5	379.0 ± 378.6 (143.0–1363.0)	205.0 (176.0–532.4)	10
Black	6	324.1 ± 156.1 (73.4–443.5)	405.9 (185.5–430.7)	4	3.4 ± 1.5 (2.4–6.4)	2.9 (2.6–3.4)	34	16.7 ± 3.2 (12.1–21.7)	16.9 (15.0–17.7)	12	1167.8 ± 280.5 (997.1–1723.9)	1034.3 (1030.4–1186.9)	31
Brown	10	325.8 ± 162.2 (65.1–613.8)	329.2 (268.8–408.6)	4	4.0 ± 3.6 (1.3–13.1)	2.4 (1.7–5.0)	40	15.9 ± 3.1 (12.7–23.1)	15.1 (14.0–15.8)	11	1017.0 ± 386.5 (149.3–1468.5)	964.0 (882.0–1351.5)	27
Parboiled	10	137.5 ± 127.2 (23.7–463.7)	89.4 (68.8–175.0)	2	2.4 ± 0.5 (1.4–3.3)	2.5 (2.1–2.7)	24	6.5 ± 6.3 (1.8–22.8)	3.9 (3.1–6.9)	5	224.9 ± 87.1 (7.1–312.4)	239.5 (211.9–283.6)	6
Red	5	216.7 ± 227.6 (51.3–545.1)	66.0 (53.7–367.2)	2	3.7 ± 1.3 (2.3–5.5)	3.4 (2.8–4.3)	37	11.8 ± 3.2 (8.7–15.5)	10.0 (9.9–15.1)	9	1241.7 ± 305.6 (1014.6–1754.3)	1086.0 (1061.5–1292.3)	33
Wild	5	294.6 ± 143.5 (83.7–421.2)	367.2 (209.6–391.5)	5	12.6 ± 2.4 (10.5–16.6)	11.7 (11.5–12.8)	126	17.9 ± 3.1 (14.0–21.8)	17.7 (16.1–20.0)	13	1043.6 ± 155.1 (873.7–1292.3)	1014.0 (977.7–1060.0)	28
White	11	114.1 ± 72.6 (16.1–278.2)	88.2 (66.9–150.1)	1	2.4 ± 0.7 (1.4–3.8)	2.4 (1.7–2.9)	24	4.5 ± 2.9 (1.4–9.9)	3.7 (2.0–5.2)	3	258.2 ± 101.4 (109.6–423.8)	234.0 (181.7–371.9)	7
Expanded	8	187.0 ± 118.0 (74.0–406.2)	170.1 (81.5–256.3)	2	2.9 ± 1.9 (1.7–7.6)	2.4 (1.8–2.7)	29	4.8 ± 2.7 (2.1–10.6)	4.4 (2.9–5.6)	3	412.0 ± 138.3 (262.2–703.4)	415.3 (305.6–444.5)	11
Flakes	12	156.3 ± 94.01 (63.1–362.6)	129.5 (107.2–158.0)	2.	3.3 ± 1.2 (2.1–6.5)	3.0 (2.6–3.7)	33	5.5 ± 5.9 (1.4–18.2)	3.3 (3.0–4.2)	4	319.8 ± 318.0 (91.7–1086.2)	200.1 (137.4–305.7)	9
Flour	6	192.1 ± 132.3 (65.7–426.3)	185.8 (67.7–221.3)	2	2.4 ± 0.5 (1.7–3.0)	2.4 (2.2–2.7)	24	8.7 ± 5.1 (4.5–18.1)	7.4 (4.7–10.3)	6	571.4 ± 416.1 (264.8–1385.1)	433.6 (333.4–578.2)	15
Pasta	7	276.8 ± 279.5 (73.5–867.0)	185.6 (85.6–355.2)	3	2.4 ± 0.7 (1.4–3.2)	2.8 (1.7–2.9)	24	3.9 ± 1.5 (2.1–6.3)	3.5 (2.4–5.6)	3	125.7 ± 59.5 (81.6–250.4)	107.7 (86.9–146.7)	3
Waffles	9	402.7 ± 118.2 (134.3–574.7)	421.6 (379.3–437.7)	5	4.9 ± 3.1 (1.6–12.3)	4.2 (3.4–5.1)	49	17.8 ± 5.1 (12.5–28.8)	17.9 (14.0–20.1)	13	1361.9 ± 283.6 (929.4–1811.5)	1383.0 (1187.4–1544.1)	36
TOTAL	99	226.3 ± 160.6 (16.1–867.0)	176.1 (85.6–362.6)	-	3.6 ± 2.8 (1.3–16.6)	2.7 (2.1–3.4)	-	9.4 ± 7.0 (1.4–28.8)	5.9 (3.4–15.1)	-	618.0 ± 498.4 (7.1–1811.5)	382.3 (211.9–1030.4)	-

X—mean, SD—standard deviation, Min—minimum, Max—maximum, Me—median, Q_1—lower quartile, Q_3—upper quartile, RVI—reference value intake, calculated per 100 g of product.

Table 4. The content of Mn, Se and Zn in rice and rice products.

The Type of the Rice and Rice Product	*n*	Mn (mg/kg)			Se (µg/kg)			Zn (mg/kg)		
		X ± SD (Min–Max)	Me (Q_1–Q_3)	% of RVI (2 mg)	X ± SD (Min–Max)	Me (Q_1–Q_3)	% of RVI (55 µg/kg)	X ± SD (Min–Max)	Me (Q_1–Q_3)	% of RVI (10 mg)
Basmati	10	10.3 ± 4.2 (0.9–16.0)	10.3 (8.7–13.3)	52	303.1 ± 129.0 (213.3–630.7)	248.1 (222.1–313.8)	55	14.5 ± 1.7 (12.4–17.8)	14.5 (13.3–15.5)	14
Black	6	27.9 ± 5.4 (22.1–37.7)	26.9 (24.9–28.9)	139	185.2 ± 25.1 (157.6–229.0)	176.7 (173.2–197.7)	34	26.0 ± 3.3 (21.5–29.8)	26.6 (22.8–28.7)	26
Brown	10	24.6 ± 7.2 (11.5–35.7)	25.1 (22.0–29.3)	123	205.5 ± 98.8 (152.1–472.0)	168.1 (164.0–172.4)	37	26.0 ± 26.5 (10.4–100.3)	18.6 (15.4–22.1)	26
Parboiled	10	6.4 ± 4.8 (0.3–15.8)	4.7 (4.2–7.0)	32	391.8 ± 190.4 (167.6–673.2)	371.5 (219.3–569.0)	71	6.7 ± 2.9 (2.4–12.9)	6.0 (5.2–6.8)	7
Red	5	38.2 ± 8.7 (31.4–53.0)	36.2 (32.7–37.5)	191	194.5 ± 9.8 (180.4–204.3)	195.6 (189.5–202.6)	35	28.1 ± 7.7 (22.0–39.4)	23.8 (22.4–33.1)	28
Wild	5	16.1 ± 2.3 (13.9–19.8)	16.0 (14.4–16.4)	81	173.0 ± 11.1 (153.9–183.1)	175.9 (175.2–176.9)	32	63.3 ± 17.2 (44.6–85.4)	62.7 (48.7–74.9)	63
White	11	13.6 ± 4.6 (8.2–22.2)	13.1 (9.6–16.3)	68	214.2 ± 34.6 (170.7–275.0)	203.6 (188.9–244.5)	39	16.3 ± 2.0 (13.7–20.3)	15.9 (14.8–17.3)	16
Expanded	8	13.8 ± 4.1 (8.8–20.1)	14.0 (9.8–16.7)	69	247.7 ± 92.2 (164.5–463.5)	229.3 (196.3–251.1)	45	16.4 ± 4.2 (10.7–22.4)	15.0 (13.6–20.7)	16
Flakes	12	12.7 ± 5.4 (7.8–26.5)	11.1 (8.8–14.8)	64	196.9 ± 26.7 (171.8–244.5)	186.8 (179.0–212.2)	36	15.8 ± 4.0 (9.4–22.6)	15.01 (13.5–18.3)	16
Flour	6	15.7 ± 9.4 (8.2–34.1)	12.1 (11.4–16.1)	78	381.2 ± 389.0 (188.7–1174.1)	236.1 (206.2–246.1)	69	15.2 ± 2.4 (12.4–19.0)	15.2 (12.9–16.6)	15
Pasta	7	7.7 ± 3.8 (4.0–15.8)	7.4 (5.8–7.6)	39	211.4 ± 26.2 (163.5–234.8)	219.7 (188.3–232.4)	38	11.3 ± 5.3 (5.5–19.7)	10.2 (6.5–16.5)	11
Waffles	9	27.4 ± 6.1 (23.8–42.7)	25.0 (24.0–27.8)	137	181.1 ± 22.2 (150.1–207.2)	187.6 (155.7–197.3)	33	19.8 ± 4.0 (12.6–26.3)	20.2 (17.3–22.0)	20
TOTAL	99	16.7 ± 10.0 (0.3–53.0)	14.1 (8.8–24.0)	-	242.9 ± 140.4 (150.1–1174.1)	200.5 (176.9–241.5)	-	19.5 ± 15.0 (2.4–100.3)	16.1 (12.9–21.7)	-

X—mean, SD—standard deviation, Min—minimum, Max—maximum, Me—median, Q_1—lower quartile, Q_3—upper quartile, RVI—reference value intake, calculated per 100 g of product.

Table 5. The statistically relevant differences in the content of the studied elements considering the type of product.

	Basmati	**Black**	**Brown**	**Parboiled**	**Wild**	**Flakes**	**Pasta**	**Waffles**
Black				$Mn^{0.001}$ $Zn^{0.001}$				
Brown	$Se^{0.01}$			$Mn^{0.001}$ $Se^{0.05}$ $Zn^{0.01}$		$Fe^{0.01}$	$Fe^{0.05}$ $Mg^{0.01}$ $Mn^{0.01}$	
Red	$Mn^{0.01}$			$Mn^{0.001}$ $Zn^{0.05}$		$Mg^{0.05}$ $Mn^{0.05}$	$Mg^{0.001}$ $Mn^{0.001}$ $Zn^{0.001}$	
Wild	$Se^{0.05}$ $Zn^{0.05}$			$Zn^{0.001}$		$Fe^{0.05}$	$Mg^{0.01}$ $Zn^{0.01}$	
White		$Fe^{0.05}$	$Fe^{0.05}$	$Se^{0.05}$	$Fe^{0.05}$			$Ca^{0.01}$ $Fe^{0.01}$ $Mg^{0.01}$
Flakes		$Mg^{0.05}$						$Ca^{0.05}$ $Fe^{0.01}$ $Mg^{0.01}$
Pasta		$Mg^{0.001}$ $Mn^{0.01}$ $Zn^{0.001}$						$Fe^{0.05}$ $Mg^{0.001}$ $Mn^{0.001}$
Waffles	$Mg^{0.001}$ $Se^{0.05}$			$Ca^{0.05}$ $Mg^{0.001}$ $Se^{0.05}$ $Zn^{0.001}$				

4. Discussion

In this investigation, we assessed the quality of 12 different groups of rice products available on the Polish market by determining the content of macro- and microelements. The results obtained by other authors are presented in Table 6.

Table 6. Content of elements in rice and rice products determined by other authors.

Type of Product [Ref.]	*n*	Element						
		Ca (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	Mg (mg/kg)	Mn (mg/kg)	Se (μg/kg)	Zn (mg/kg)
Brown Rice								
[16]	9	Min–Max: 87.1–114	Min–Max: 1.3–4.6	Min–Max: 13.3–14.5	Min–Max: 160–1630	Min–Max: 7.6–11.8	-	-
[17]	6	-	X = 3.1	X = 11.5	-	X = 28.9	X = 104	X = 22.5
[18]	11	X = 64±9	X = 1.6 ± 0.4	X = 14.0 ± 2.1	X = 1064 ± 87	X = 21.5 ± 4.4	X = 30 ± 20	X = 15.9 ± 2.3
[19]	16	X = 104 ± 37.9	X = 3.0 ± 1.1	X = 20.1 ± 7.8	X = 1205 ± 335	X = 26.5 ± 12.2	X = 131 ± 57	X = 20.2 ± 2.73
[20]	33	X = 72.6 ± 32.6 (35.6–211.5)	X = 5.5 ± 5.4 (Min–Max: 2.2–29.1)	X = 32 ± 26.1 (Min–Max: 12.1–119.1)	X = 1140 ± 214 (Min–Max: 761–1550)	X = 20.1 ± 10.5 (Min–Max: 12.4–74.4)	X = 41 ± 57 (Min–Max: 5–300)	X = 18.8 ± 4.3 (Min–Max: 13.9–34)
[21]	51	-	X = 2.35 (Min–Max: 1.4–3.9)	X = 18.6 (Min–Max: 10.0–65.2)	-	X = 15.5 (Min–Max: 8.2–24.2)	-	X = 21.0 (Min–Max: 9.0–29.4)
[22]	51	-	X = 4.4 (Min–Max: 0.9–6.5)	-	-	X = 20 (Min–Max: 10–34)	X = 39 (Min–Max: 15–80)	X = 28 (Min–Max: 20–36)
Our results	10	X = 325.8 ± 162.2 (Min–Max: 65.1–613.8)	X = 4.0 ± 3.6 (Min–Max: 1.3–13.1)	X = 15.9 ± 3.1 (Min–Max: 12.7–23.1)	X = 1017.0 ± 386.5 (Min–Max: 149.3–1468.5)	X = 24.6 ± 7.2 (Min–Max: 11.5–35.7)	X = 205.5 ± 98.8 (Min–Max: 152.1–472.0)	X = 26.0 ± 26.5 (Min–Max: 10.4–100.3)
White Rice								
[17]	5	-	X = 2.3	X = 3.7	-	X = 7.8	X = 92	X = 13.1
[18]	56	X = 32 ± 18	X = 1.8 ± 0.6	X = 6.8 ± 1.5	X = 225 ± 63	X = 1.8 ± 0.6	X = 200 ± 190	X = 13.5 ± 3.4
[19]	9	X = 127 ± 141 X = 37.7 ± 9.1	X = 1.7 ± 0.6	X = 22.3 ± 37.9	X = 371 ± 127	X = 10.5 ± 3.7	X = 108 ± 66	X = 15.6 ± 1.9
[20]	21	(Min–Max: 18.6–63)	X = 3.1 ± 2.0 (Min–Max: 0.9–8.1)	X = 7.9 ± 2.9 (Min–Max: 3.6–17.3)	X = 259 ± 44 (Min–Max: 191–341)	X = 9.4 ± 1.7 (Min–Max: 7.0–12.7)	X = 40 ± 35 (Min–Max: 13–137)	X = 14.7 ± 1.8 (Min–Max: 12–17.7)
Our results	11	X = 114.1 ± 72.6 (Min–Max: 16.1–278.2)	X = 2.4 ± 0.7 (Min–Max: 1.4–3.8)	X = 4.5 ± 2.9 (Min–Max: 1.4–9.9)	X = 258.2 ± 101.4 (Min–Max: 109.6–423.8)	X = 13.6 ± 4.6 (Min–Max: 8.2–22.2)	X = 214.2 ± 34.6 (Min–Max: 170.7–275.0)	X = 16.3 ± 2.0 (Min–Max: 13.7–20.3)
Parboiled Rice								
[18]	13	X = 370 ± 272	X = 2.0 ± 0.6	X = 8.6 ± 1.9	X = 370 ± 272	X = 5.6 ± 1.3	X = 100 ± 50	X = 6.1 ± 1.4
Our results	10	X = 137.5 ± 127.2 (Min–Max: 23.7–463.7)	X = 2.4 ± 0.5 (Min–Max: 1.4–3.3)	X = 6.5 ± 6.3 (Min–Max: 1.8–22.8)	X = 224.9 ± 87.1 (Min–Max: 7.1–312.4)	X = 6.4 ± 4.8 (Min–Max: 0.3–15.8)	X = 391.8 ± 190.4 (Min–Max: 167.6–673.2)	X = 6.7 ± 2.9 (Min–Max: 2.4–12.9)
Wild Rice								
[18]	6	X = 238 ± 170	X = 3.3 ± 1.9	X = 7.8 ± 1.2	X = 561 ± 170	X = 5.5 ± 0.8	X = 120 ± 40	X = 24.7 ± 4.6
Our results	5	X = 294.6 ± 143.5 (Min–Max: 83.7–421.2)	X = 12.6 ± 2.4 (Min–Max: 10.5–16.6)	X = 17.9 ± 3.1 (Min–Max: 14.0–21.8)	X = 1043.6 ± 155.1 (Min–Max: 873.7–1292.3)	X = 16.1 ± 2.3 (Min–Max: 13.9–19.8)	X = 173.0 ± 11.1 (Min–Max: 153.9–183.1)	X = 63.3 ± 17.2 (Min–Max: 44.6–85.4)
Rice								
[23]	9 *	X= 84.5	X = 1.1	X = 20.7	-	-	-	X = 23.4
[24]	56	-	X = 3.3 ± 0.9 (min-Max:0.7–6.9)	X = 39.4 ± 17.6 (Min–Max: 11.8–90.6)	-	X = 4.4 ± 1.4 (Min–Max: 2.3–8.4)	X = 280 ± 200 (Min–Max: 0–630)	X = 9.8 ± 2.8 (Min–Max: 6.3–20.3)
[25]	23	X = 71.5 ± 7.3	X = 3.7 ± 0.2	-	-	X = 8.5 ± 0.5	X = 401 ± 92	X = 10.2 ± 0.3
[26]	30	-	X = 2.0 ± 0.5	-		X = 4.7 ± 0.6	X = 25.6 ± 10	X = 13.2 ± 2.1
Our results (rice in total)		X = 226.3 ± 160.6 (Min–Max: 16.1–867.0)	X = 3.6 ± 2.8 (Min–Max: 1.3–16.6)	X = 9.4 ± 7.0 (Min–Max: 1.4–28.8)	X = 618.0 ± 498.4 (Min–Max: 7.1–1811.5)	X = 16.7 ± 10.0 (Min–Max: 0.3–53.0)	X = 242.9 ± 140.4 (Min–Max: 150.1–1174.1)	X = 19.5 ± 15.0 (Min–Max: 2.4–100.3)
Rice Flour								
[17]	4	-	X = 2.4	X = 31.2	-	X = 22.5	X = 117	X = 14.9
Our results	6	X = 192.1 ± 132.3 (Min–Max: 65.7–426.3)	X = 2.4 ± 0.5 (Min–Max: 1.7–3.0)	X = 8.7 ± 5.1 (Min–Max: 4.5–18.1)	X = 571.4 ± 416.1 (Min–Max: 264.8–1385.1)	X = 15.7 ± 9.4 (Min–Max: 8.2–34.1)	X = 381.2 ± 389.0 (Min–Max: 188.7–1174.1)	X = 15.2 ± 2.4 (Min–Max: 12.4–19.0)

Min—minimum, Max—maximum, X—mean, *—every brand with five replications.

Compared to other plant products considered to be sources of Ca (such as legumes), the Ca content in our study material was generally low; however, most of the other authors [16,19,20,23,25] observed even lower Ca levels than that measured in our samples (Tables 3 and 6). Only Pinto et al. determined in parboiled rice a higher Ca level than that in this research [18]. The parboiling process increases the Ca content due to specific conditions, such as soaking time and proper temperature [27]. The tested products could not be regarded as a source of Ca in the diet of European adults (Table 3). Insufficient Ca intake is still a worldwide problem. Taking into account Ca intake with a whole day diet, most of the available research determines the insufficient intake of this nutrient [5,28]. In the study by Grygorieva et al., among Ukrainian adults, only 7.8% of subjects consumed more than 1200 mg of Ca per day [29]. The problem of inadequate Ca consumption amid Spanish adolescents and elderly people population was observed as well [30]. Considering the daily intake levels, Kashian and Fathivand observed that rice consumption fulfilled the recommended dietary allowances (RDA) norm within 1% of the Iranian population [23]. Similar observations were found in the study conducted by Pinto et al. among the Iberian (Portugal and Spain) population; daily Ca requirements by rice consumption were met from 0.2% to 1.3% of the RDA norm [18]. Other Spanish researchers also reported that daily Ca intake through rice consumption ranged from 0.6% of RDA norm for white rice to 1.07% of RDA for brown rice. However, when brown rice was replaced by white rice, the daily Ca intake was reduced by 47% [16].

The Cu levels measured in this investigation are in agreement with those reported by other authors [16–22,24–26]. Taking into account the recommendations of Cu intake, rice products available on the Polish market could be regarded as a source of this element in the daily diet of European adults (Table 3). Regarding rice consumption, observations were made by Antoine et al. that among the Jamaican population, polished rice intake fulfilled daily Cu requirements in 13%, while unprocessed grains in 23% [19]. Among Malawian adults, daily rice intake covered 19% of the RDA, while amid the Nigerian population, 31% [20,25]. Lower daily Cu intake was reported for Iberian populations and ranged from 6.4% to 13% of the RDA norm [18]. Jo and Todorov demonstrated that in rice grains, Cu is concentrated mainly in the bran and outer layers of the endosperm. When brown rice was polished to white rice (removal 18% of grain weight), the Cu content decreased by nearly 20% from 2.29 mg/kg to 1.91 mg/kg [31]. Moreover, Hensawang et al. reported that extra thorough rice polishing reduced Cu content by 35% [32]. Ortiz and Camara-Martos determined Cu bioaccessibility through solubility and dialyzability assays among eight of the most consumed rice varieties in Argentina and Spain. The bioaccessibility ranged from 24% to 80%, while dialyzability ranged from 4% to 41% [33]. In the study by Babaali et al., among the Iranian population, the average daily Cu intake exceeded the estimated average requirements (EAR) and was determined at 3.8 mg; fresh fruit and vegetables as the main sources (31%) of this element were indicated [34]. More than three times lower dietary Cu consumption (1.2 mg/day) was reported in the investigation conducted among Japanese adults. The authors observed that white rice accounted for approximately 20–30% of the total intake of this element [35].

Considering the Fe content in rice samples, some other authors [16–18] reported comparable results to ours (Tables 3 and 6). However, higher Fe levels were also frequently reported [19–21,23,24]. None of the subgroups tested can be a source of Fe for the adult European population (Table 3). Women of childbearing age are at risk of inadequate Fe intake due to high body requirements for this element. Considering Fe intake with whole day diet among Spanish adults, only 17% of women had sufficient intake, while among men, it was 57.3% of participants [36]. Similar observations were reported among Polish adolescents; females had significantly lower Fe intake than males [37]. On the other hand, both white and brown rice fulfilled daily RDA for men in Jamaica at 20% and 18%, while for women it was 8.8% and 8%, respectively [19]. Among the Iranian population, rice consumption covered the recommended daily intake in 14% for women and 31% for men [23]. Fe in rice grains is unevenly distributed and located mainly in the

pericarp and aleurone layers. Five times higher Fe concentration was observed in the dorsal compared to the ventral side of the bran layer. The polishing process (18% removal of grain weight) reduced Fe content in brown rice from 10.4 mg/kg to 3.6 mg/kg in white rice; therefore 66% of Fe was removed [31]. Besides the total content of Fe in rice, the presence of compounds negatively affecting its bioavailability (such as phytic acid, polyphenols, tannins and dietary fiber) should be taken into account. Average Fe solubility was within the range of 80% in rice grains, except for brown rice. The lowest bioaccessible percentage, despite the high Fe content, was a result of a high concentration of the antinutritional factors mentioned above [33].

The Mg levels determined in our research (Table 3) were close to those reported in other investigations [16,18,20,24]. Except for six groups of products (basmati, expanded, parboiled, white rice, flakes and pasta), the remaining rice products can be a source of Mg for European adults (Table 3). Taking into account the daily rice consumption, Portuguese and Spanish citizens fulfilled the RDA norm from 2.4% to 11.8% among women and between 1.8% and 9% among men. Amid the Jamaican population, white rice consumption covered Mg daily requirements in 8.2% in females and 6.3% in males, while brown rice in 27% and 20%, respectively [19]. Cano-Lamarid et al. observed that the replacement of brown rice with white rice in the diet may result in a reduction in daily Mg intake by 70% [16]. Daily Mg intake was generally adequate in research conducted by Schiefermeier-Mach et al.; however, in nearly one-third of participants, the coverage of requirements was not met [38]. Similar results were observed among the Iranian population—22.4% of the study group had an Mg intake lower than the EAR norm [34].

A lower Mn content than found in this investigation was reported by Pinto et al. in the case of white and wild rice, and by Halder et al. also, in white rice [18,24], whereas most of the other researchers determined similar levels of Mn in rice samples [16,17,19–22,25,26]. Every subgroup of the rice products tested can be a rich source of Mn in the diet of the adult European population (Table 4). One portion (300 g) of red rice reached the TUIL of Mn in our investigation. Moreover, when another foodstuff consumed daily is added, there is a potential risk of elevated Mn levels in the body, which could pose negative effects. The investigation by Choi and Bae among Korean adults demonstrated that Mn intake with diet covered the adequate intake (AI) norm in 103% for men and 110% for women and the main sources of this element were cereal and cereal products [39]. Regarding the daily intake, in Antoine's et al. investigation, white rice consumption fulfilled the recommended level of intake for women at 41% and men at 32%, while brown rice at 105% and 82%, respectively [19]. Pinto et al. estimated that the AI norm for the Iberian females through rice consumption was covered from 10.8% to 42.5% and from 8.5% to 33.3% for Iberian males [18]. The total content of Mn in the investigation conducted by Ortiz and Camara-Martoz ranged from 2.5 mg/kg to 14 mg/kg. Taking into account the bioaccessibility, the concentration of soluble Mn varied from 0.35 mg/kg to 2.52 mg/kg, while the dialyzable fluctuated from 0.15 mg/kg to 0.64 mg/kg. Moreover, the authors observed a statistically significant negative interaction between the content of soluble Mn and fiber in the studied grains [33]. Hensawang et al. found that the polishing process decreased the Mn concentration by 62% [32]. The content in unpolished and polished grains differed significantly ($p < 0.01$, $r = -0.747$). On the other hand, Jo and Todorov reported lower Mn reduction (43%) during the polishing process. The Mn in rice grain, similar to Fe, is localized mainly in the pericarp and aleurone layers; however, this trace element is also present in the endosperm. In the dorsal, compared to the ventral, side of the bran layer, a five times higher Mn concentration was observed [31].

Considering Se levels in rice products, our results were higher than reported by most of the other researchers [17–20,22,26]. Only Halder et al. measured a fairly similar content, while Aderide et al. determined greater amounts of Se in tested rice [24,25]. Due to considerably high Se levels, every subgroup of the rice products tested can be regarded as a source of this trace element for European adults (Table 4). Cereals and meat were indicated as the main sources of Se in the diet of Italian adults. The daily level of Se consumption was

sufficient; for women, it was estimated at 65 μg/day and for men at 67 μg/day [40]. Similar results were observed among Belgian adults [41]. Taking into account rice consumption, among the Brazilian population, rice intake covered 5% of the daily Se requirement [25]. For Jamaican adults, brown rice consumption covered 17% of the daily norm, while white rice covered 14% [19]. In the research conducted by Jo and Todorov, the polishing process slightly decreased Se content in whole grain rice from 150 μg/kg to 144 μg/kg in white rice. Additional analysis showed that Se is evenly distributed through the grain [31].

The Zn content in this study (Table 4) was comparable to the results obtained by most of the other authors cited in Table 6 [17–21,23]. In three studies, the measured Zn levels were lower than observed in our samples [24–26]. Only Rothenberg et al. reported higher Zn levels [22]. Except for basmati and parboiled rice as well as pasta, the remaining studied products could be a source of Zn for the adult European population (Table 4). In the investigation of Pinto et. al, consumption of rice fulfilled the RDA norm by 1.8% to 10.9% [18]. Fairly similar fulfilment of the RDA norm (9%) was observed amid Nigerian women [25]. During the polishing process, the content of Zn was reduced by 23% (from 18.3 mg/kg to 14.1 mg/kg). Generally, Zn is distributed homogeneously through the grain; however, the bran layer contains higher average Zn levels (20–80 mg/kg) compared to the endosperm (13 mg/kg) [31]. The high content of antinutritional components in bran impaired Zn bioaccessibility from rice grains. In the study by Ortiz and Camara-Martoz, the total Zn level ranged from 8.8 mg/kg to 13 mg/kg and the following soluble and dialyzable contents were reported: 0.9–3.3 mg/kg and 0.2–1.3 mg/kg, respectively. Thus, Zn bioaccessibility was quite low—the solubility did not exceed 15%—while dialyzability was lower than 3%. It was observed that vegetable proteins were negatively correlated ($p < 0.05$, $r = -0.409$) with Zn bioaccessibility [33]. Considering the overall daily diet, among Chinese adults, over one-third are at risk of Zn deficiency due to intake below the EAR norm. Grain consumption contributed to nearly 40% of total dietary Zn intake [42]. On the other hand, among American adults, daily Zn intake above RDA norm was reported [43].

To the best of our knowledge, there are limited data regarding the TUIL for elements consumed with diet. The risk of Ca intake with food products reaching TUIL is very low [44]. In a large European cohort study, exceedances of TUIL considering Fe (among women and men) and Mg (among men) were reported. The risk of over intake was observed among those who consumed dietary supplements [45]. No nutrient intakes with a diet above the TUIL were reported among the Canadian population. However, study participants who took dietary supplements were at risk of overly high Mg and Zn intake [46]. In turn, our analyses showed that there could be a potential risk of over intake of Mn through the whole day diet (even without supplementation). Therefore, we see a great need to conduct research focused on this topic.

5. Conclusions

Taking all of the above described into account, our study revealed that all of the studied subgroups of rice products available on the Polish market can be regarded as a source of Cu, Mn, and Se, while most of the products can be a source of Mg and Zn in the diet of the adult European population. Therefore, this fact should be highlighted in nutritional education. Considering the tolerable upper intake level of the studied elements, the tested products could be regarded as safe to consume. However, we see a great need to assess the daily intake of Mn with all products consumed in the diet due to the possible risk of excessive intake.

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Macroelements and Trace Elements Content in Brine-Canned Mackerel (*Scomber colias*) Subjected to High-Pressure Processing and Frozen Storage

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Abstract: This study analysed the effect of prior high-pressure processing (HPP; 200–600 MPa, 2 min), freezing (−30 °C, 48 h), and frozen storage (−18 °C, 6 months) on the macroelement and trace element content in brine-canned mackerel (*Scomber colias*). Most elements (*Na*, *Ca*, *Ba*, *Mn*, *Fe*, *Cu*, *Cd*, *Sn*, *As*, *S*, and *Se*) showed an increased ($p < 0.05$) presence in mackerel muscle canned after freezing. A content increase ($p < 0.05$) was also observed for *Na* and *Sn* if prior frozen storage was also applied; on the contrary, *Ca*, *Ba*, *Mn*, *Fe*, *Cd*, *S*, and *Se* showed a content decrease ($p < 0.05$) as a result of such storage. Freezing, frozen storage, and canning led to lower values ($p < 0.05$) in canned fish for *K*, *Mg*, *Pb*, and *P*. Prior HPP led to relevant content decreases ($p < 0.05$) for *K*, *Mg*, *Ca*, *Ba*, *Mn*, *Fe*, *Pb*, and *P* contents in fish canned after the freezing step; HPP provoked additional decreases ($p < 0.05$) in *Ca*, *Ba*, and *Mn* levels in samples corresponding to 6-month frozen storage. On the contrary, prior HPP led to marked increases ($p < 0.05$) for *Cd*, *S*, and *Se* contents in all canned samples. Content changes are explained on the basis of modifications of other constituents and liquor losses from muscle.

Keywords: chub mackerel; high-pressure processing; freezing; frozen storage; canning; macroelements; trace elements; protein denaturation; liquor loss: packaging medium

1. Introduction

Marine products are known to be highly perishable. Consequently, a rapid and efficient processing and storage have to be accomplished after capture or harvest to retain the initial quality. One such process is canning. The heat treatment involved in it can substantially alter the nature of the raw starting material and provide a food product with different characteristics [1,2]. Remarkably, both enzymes and bacteria should be permanently inactivated, giving rise to a safe and durable food, provided reinfection does not occur and no negative interaction with the container is produced. Unfortunately, most marine species employed in canneries have to be stored previously. Therefore, most quality problems with canned fish can be related to the quality loss of the raw material employed [3,4].

In agreement with the short shelf life of chilled fish, freezing followed by frozen storage has been employed widely as a prior storage condition to canning process. Nevertheless, if long storage periods and/or relatively high temperatures are applied, quality assessment demonstrates that fish deterioration is produced during frozen storage since detrimental changes associated especially with lipids and proteins can occur [5–7]. Consequently, several complementary technologies have been applied to inhibit deterioration during fish frozen storage. Among them, high-pressure processing

(HPP) has shown to retain the nutritional and sensory characteristics of seafood and lead to the inactivation of microbial growth and endogenous enzymes (hydrolytic and oxidative), so that an extended shelf life in seafood is achieved [8–10]. Concerning frozen seafood, HPP has shown potential industrial application as assisting freezing and thawing processing [11,12]. Furthermore, previous HPP has led to marked inhibition of damage mechanisms such as lipid hydrolysis and oxidation, and trimethylamine oxide breakdown during frozen storage of fatty and lean fish species [13–15]. Reports addressing the employment of HPP as assisting thermal treatment can be considered scarce [16,17]; concerning canned seafood, a previous study has recently shown a strong effect of pressure level on the subsequent development of lipid hydrolysis and oxidation of canned mackerel [18].

Marine fish live in a medium rich in mineral salts. Thus, the majority of the macroelements and trace elements considered essential for biological processes can be found in marine species [19–21]. However, presence of toxic trace elements has led to some health risks in commercially available fish and fish products [22,23]. Marine organisms have shown to accumulate minerals from the diet and deposit them in their skeletal tissues and organs. Furthermore, interaction of the different elements with other fish constituents has shown a great dependence on their chemical characteristics. Thus, alkali (*Na* and *K*) and alkali earth (*Mg* and *Ca*) elements have shown to be present in the cellular medium as chlorides, sulphates, or organic salts such as citrates, lactates, or pyruvates. On the contrary, transition metals (*Fe*, *Cu*, etc.) and non-positive elements (*S*, *P*, etc.) have shown to be strongly bound to other muscle constituents and give rise to a wide number of functional molecules [24,25]. Different factors such as species, tissue, maturation degree, nourishment source, season, environment, and processing may influence the concentration of elements in fish [26–28]. Remarkably, previous reports include abundant information on chemical changes related to constituents such as proteins and lipids during processing and storage of fish species. However, research focused on changes on mineral content during fish processing can be considered scarce.

The current research focuses on the mineral content of canned fish. In it, the effect of prior HPP (200–600 MPa, 2 min), freezing (−30 °C, 48 h), frozen storage (−18 °C, 6 months), and canning was analysed on the chemical elements content (macroelements and trace elements; essential and toxic) in brine-canned Atlantic Chub mackerel (*Scomber colias*). To the best of our knowledge, previous research related to the HPP effect on mineral content in seafood in general can be considered very scarce, and inexistent in the case of canned seafood.

2. Materials and Methods

2.1. Materials

Mackerel specimens (54 individuals; length and weight ranges: 26.3 ± 1.8 cm and 166 ± 9 g, respectively) were caught near the Galician Atlantic coast, acquired at Vigo harbour (North-Western Spain) and transported to the laboratory. Throughout this process (6–8 h), the fish were maintained in ice.

Nitric acid (Hiperpur; TMA) was obtained from PanReac AppliChem ITW Reagents (Barcelona, Spain). Element standards were obtained from Sigma-Aldrich (Steinheim, Germany). Other chemicals employed were reagent grade (Sigma-Aldrich, Madrid, Spain).

2.2. Initial Raw Fish, HPP, Freezing, and Frozen Storage

Six fish individuals were taken and distributed into three groups (two specimens per group). Such specimens (initial raw fish) were filleted, the white muscle being analysed independently within each group ($n = 3$).

The remaining specimens were placed in flexible polyethylene bags (8 bags; six individuals per bag), vacuum-sealed at 150 mbar (Vacuum Packaging Machine Culinary, Albipack, Águeda, Portugal) and distributed into four batches (2 bags in each batch). Bags corresponding to one of such batches were directly kept at −30 °C for 48 h (freezing treatment) and labelled as CT batch (control fish). Bags

corresponding to the other three batches were subjected to HPP (200, 400, and 600 MPa for 2 min, respectively) in a 55-L high pressure unit (WAVE 6000/55 HT; NC Hiperbaric, Burgos, Spain). With this purpose, water was employed as pressurising medium at 3 MPa·s^{-1} yielding 67, 133, and 200 s as the come up times, respectively; decompression time was less than 3 s. After pressurisation, all bags were kept at −30 °C for 48 h (freezing process).

Once the freezing step was accomplished, fish belonging to one bag of each batch (CT, 200-, 400- and 600-MPa batches) were thawed (4 °C, overnight) and then employed for the canning process leading to month-0 canned fish (M-0 samples). The remaining bags (1 bag per batch) were kept at −18 °C for 6 months. At this time, fish packaged in all bags were thawed overnight at 4 °C and then employed for the canning process leading to month-6 canned fish (M-6 samples).

2.3. Canning and Sampling Procedure

The canning process was carried out on thawed fish corresponding to all batches. Thus, thawed fish were filleted, 45-g portions (from one fish individual and without including the tail part) being placed in flat rectangular cans (105 × 60 × 25 mm; 150 mL). Then, cans were filled with brine solution (2% *w*/*v*), vacuum-sealed and subjected to sterilisation process (125 °C, 45 min; F_0 = 10 min) in a horizontal steam retort (Conservas Cerqueira, S. A., Vigo, Spain); a prior come-up time of 20 min was employed. Then, steam was cut off, the remaining steam was flushed away with air, and cans cooling was carried out at reduced pressure.

After a 3-month storage at room temperature (20 °C), the cans were opened, and the liquid phase was carefully drained off gravimetrically and filtered through a filter paper. Fish white muscle was separated, wrapped in filter paper, and used for analysis. For each batch, the fish white muscle corresponding to two cans was pooled together to carry out the chemical analyses. Three replicates (n = 3) of each batch were analysed.

A 3-month canned storage at room temperature was employed according to recommendation of canning manufacturers in order to obtain optimal palatability in canned products [29].

2.4. Moisture Determination

Moisture content of fish samples was assessed as the weight difference in homogenised muscle (1–2 g) before and after 4 h at 105 °C [30]. Results were expressed as g·kg^{-1} muscle.

2.5. Mineral Analysis

The content of sixteen chemical elements (*Na*, *K*, *Mg*, *Ca*, *Ba*, *Mn*, *Fe*, *Co*, *Cu*, *Cd*, *Sn*, *Pb*, *P*, *As*, *S*, and *Se*) was analysed according to the following procedure based on EPA 3050B [31]. About 1 g of ground sample was put into a digestion flask with 9 mL of 69% nitric acid (TMA) Hiperpur, 3 mL of H_2O_2 (for ultratrace analysis), and 3 mL of Milli-Q water. Samples, plus four blanks and four samples of certified reference material, were digested in a microwave oven (Mars-Xpress CEM Corp. Matthews, NC, USA). Samples were completely digested, and solutions were transferred to 50 mL flasks. Handling of samples was carried out inside a clean ISO 5 laminar flow cabinet (Cruma 670 FL, Barcelona, Spain). The sixteen aforementioned elements were analysed by ICP-MS (inductively coupled plasma-mass spectrometry) using an Agilent 7900 equipment (Agilent Technologies, Inc., Santa Clara, CA, USA). The quantification was carried out by external calibration with element standards traceable to NIST (National Institute of Standards and Technology) standards. The limits of detection were calculated with respect to the standard deviation of the blanks (LD = 3·SD blanks). Procedural blanks always accounted for <1% of element concentrations in the samples. Accuracy of the analytical procedures was ensured using DORM-2 certified reference material, prepared by the National Research Council of Canada (NRCC), as the quality control material (Table 1). The reference values of macroelements and *Ba* in DORM-2 were reported by Engström et al. [32]. Results were calculated as g·kg^{-1} dry muscle (macroelements) and as mg·kg^{-1} dry muscle (trace elements).

Table 1. Accuracy control of the analytical procedures for the assessment of macroelements and trace elements *.

Element	Certified	Measured	Unit
MACROELEMENTS			
Ca	0.62 ± 0.05	0.62 ± 0.09	$g \cdot kg^{-1}$
K	18.9 ± 1.1	17.0 ± 0.5	"
Mg	1.05 ± 0.05	1.15 ± 0.05	"
Na	5.06 ± 0.07	5.72 ± 0.17	"
P	9.9 ± 0.1	10.6 ± 0.5	"
S	8.9 ± 0.5	8.5 ± 0.2	"
TRACE ELEMENTS			
As	18.0 ± 1.1	17.3 ± 1.8	$mg \cdot kg^{-1}$
Ba	2.34 ± 0.03	2.4 ± 0.3	"
Cd	0.043 ± 0.008	0.038 ± 0.002	"
Co	0.182 ± 0.031	0.16 ± 0.02	"
Cu	2.34 ± 0.16	1.92 ± 0.23	"
Fe	142 ± 10	105 ± 15	"
Pb	0.065 ± 0.007	0.047 ± 0.007	"
Mn	3.66 ± 0.34	3.02 ± 0.29	"
Se	1.40 ± 0.09	1.41 ± 0.12	"
Sn	0.023 ± 0.001	0.026 ± 0.009	"

* Data expressed as mean value ± standard deviation ($n = 4$). Certified reference material was DORM-2 (National Research Council of Canada (NRCC)), where macroelements and *Ba* values were reported by Engström et al. [32].

2.6. Statistical Analysis

Results were subjected to the ANOVA method to analyse differences resulting from the effect of the different steps carried out (HPP, freezing, frozen storage, and canning). Comparison of average values was performed using the least-squares difference (LSD) method. In all cases, analyses were carried out using the PASW Statistics 18 software for Windows (SPSS Inc., Chicago, IL, USA); differences among batches were considered significant for a confidence interval at the 95% level ($p < 0.05$).

3. Results

3.1. Changes in Alkali (Na and K) and Alkaline Earth (Mg, Ca, and Ba) Metals Content

Comparison between initial raw fish and M-0 canned samples showed an important increase ($p < 0.05$) in *Na* content resulting from the freezing step followed by the canning process (Table 2). This result could be observed both for high-pressure treated and untreated fish. Furthermore, average values of M-0 mackerel showed to be increased in all batches if a 6-month frozen storage was also applied (i.e., M-6 canned fish), such increases being significant ($p < 0.05$) for CT and 400-MPa batches. Comparison among batches led to scarce differences. Therefore, a definite trend about the effect of prior pressure level could not be concluded ($p > 0.05$).

Concerning the *K* and *Mg* levels in canned fish, HPP, freezing, and canning showed an opposite effect than in the case of Na (Table 2). Thus, a general marked decrease ($p < 0.05$) could be outlined in M-0 samples corresponding to all batches when compared with the starting raw fish; all such values were significantly decreased ($p < 0.05$) if a 6-month storage was also employed. Prior pressure applied showed a decreasing effect on both elements content for M-0 mackerel. Hence, lower values ($p < 0.05$) were observed in canned fish corresponding to 400- and 600-MPa batches. However, significant differences ($p > 0.05$) were not detected among canned samples if a 6-month storage was taken into account, although fish corresponding to the control batch revealed the highest average values for both essential elements.

Table 2. Determination of different alkali (*Na* and *K*) and alkaline earth (*Mg* and *Ba*) metal elements contents * in initial raw fish and canned mackerel subjected to different processing conditions **.

Element	Frozen Storage Time (Months)	High-Pressure Processing (HPP) (MPa)			
		CT	**200**	**400**	**600**
Na ($g \cdot kg^{-1}$ dry muscle)	Initial raw fish	2.31 A (0.04)	2.31 A (0.04)	2.31 A (0.04)	2.31 A (0.04)
	0	5.07 abB (0.10)	5.14 bB (0.06)	4.95 aB (0.10)	4.98 abB (0.28)
	6	5.48 aC (0.22)	5.46 aB (0.26)	5.63 aC (0.24)	5.40 aB (0.19)
K ($g \cdot kg^{-1}$ dry muscle)	Initial raw fish	3.46 C (0.19)	3.46 C (0.19)	3.46 C (0.19)	3.46 C (0.19)
	0	1.53 bB (0.05)	1.55 bB (0.12)	1.37 aB (0.02)	1.29 aB (0.10)
	6	1.10 aA (0.13)	1.05 aA (0.11)	1.05 aA (0.03)	1.00 aA (0.05)
Mg ($g \cdot kg^{-1}$ dry muscle)	Initial raw fish	0.51 C (0.02)	0.51 C (0.02)	0.51 C (0.02)	0.51 C (0.02)
	0	0.18 bB (0.01)	0.19 bB (0.01)	0.16 aB (0.00)	0.15 aB (0.01)
	6	0.14 aA (0.01)	0.13 aA (0.00)	0.13 aA (0.00)	0.13 aA (0.01)
Ba ($mg \cdot kg^{-1}$ dry muscle)	Initial raw fish	0.054 B (0.003)	0.054 B (0.003)	0.054 B (0.003)	0.054 B (0.003)
	0	0.085 cC (0.03)	0.058 bB (0.06)	0.047 bB (0.007)	0.017 aA (0.004)
	6	0.042 cA (0.007)	0.039 cA (0.003)	0.026 bA (0.005)	0.015 aA (0.002)

* Mean values of three replicates ($n = 3$). Standard deviations are indicated in brackets. ** In each row, different lower-case letters (a–c) denote significant differences ($p < 0.05$) as a result of HPP. In each column, capital letters (A–C) denote significant differences ($p < 0.05$) as a result of freezing and frozen storage.

Evolution of *Ca* content (Figure 1) provided a similar response than that of *Ba* (Table 2) to the different processing steps included in the current study. Thus, comparison between initial raw fish and CT canned fish corresponding to month-0 storage showed a marked increase ($p < 0.05$) as a result of HPP, freezing, and canning. However, content on both elements provided a significant decrease ($p < 0.05$) in CT canned samples as a result of including a 6-month frozen storage period. Remarkably, *Ca* average levels in M-6 fish were higher than in initial raw fish (except for 600-MPa batch), while levels of *Ba* in canned samples were lower ($p < 0.05$) than in starting fish for all batches. On the other side, a marked effect can be signalled for prior pressure on the content of both elements. Thus, a significant decrease ($p < 0.05$) was implied by increasing the prior pressure applied in samples corresponding to M-0 and M-6 conditions. For both elements, canned fish corresponding to 600-MPa batch showed lower values ($p < 0.05$) than their counterpart samples corresponding to the other three batches.

3.2. Changes in Transition Metals Content (Mn, Fe, Co, Cu, and Cd)

In the case of *Mn*, comparison between initial raw fish and canned fish corresponding to month-0 frozen storage showed a significant increase ($p < 0.05$), except for canned muscle previously submitted to a 600-MPa treatment that showed a significant decrease ($p < 0.05$) (Figure 2). Furthermore, comparison between average values of M-0 and M-6 canned samples provided a decreasing effect of the prior frozen storage period. This effect was significant ($p < 0.05$) in the case of CT and 400-MPa batches.

A strong decreasing effect ($p < 0.05$) on the content of this essential element could be accorded to prior pressure processing. Thus, for both M-0 and M-6 canned samples, a progressive decrease ($p < 0.05$) by increasing the pressure level was proved.

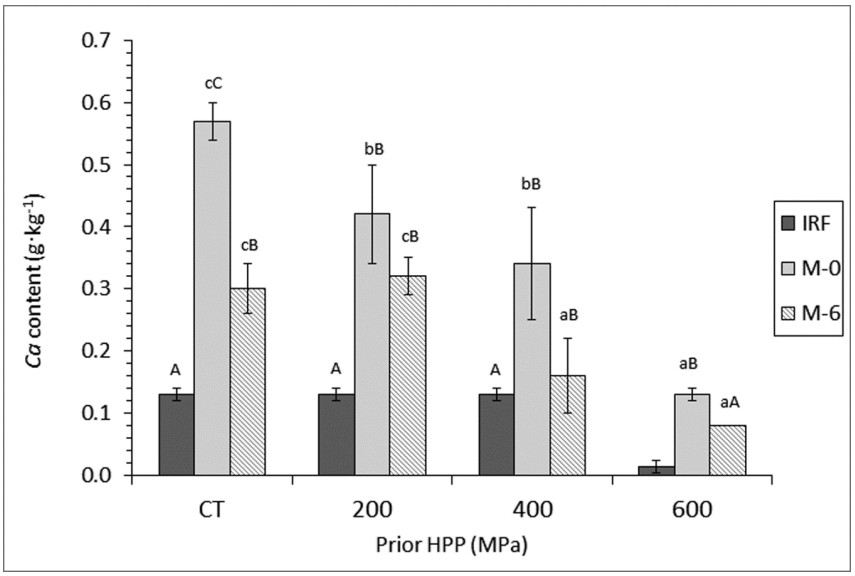

Figure 1. Determination of *Ca* content (g·kg^{-1} dry muscle) * in initial raw fish (IRF) and canned mackerel subjected to high-pressure processing (HPP), freezing, and frozen storage **. * Mean values of three replicates ($n = 3$). Standard deviations are indicated by bars. For each frozen storage time (0 or 6 months), different lower-case letters (a–c) denote significant differences ($p < 0.05$) as a result of HPP. For each HPP condition, capital letters (A–C) denote significant differences ($p < 0.05$) as a result of frozen storage time. ** Sample name abbreviations: CT (control batch; without HPP), M-0 (fish canned after freezing) and M-6 (fish canned after a 6-month frozen storage).

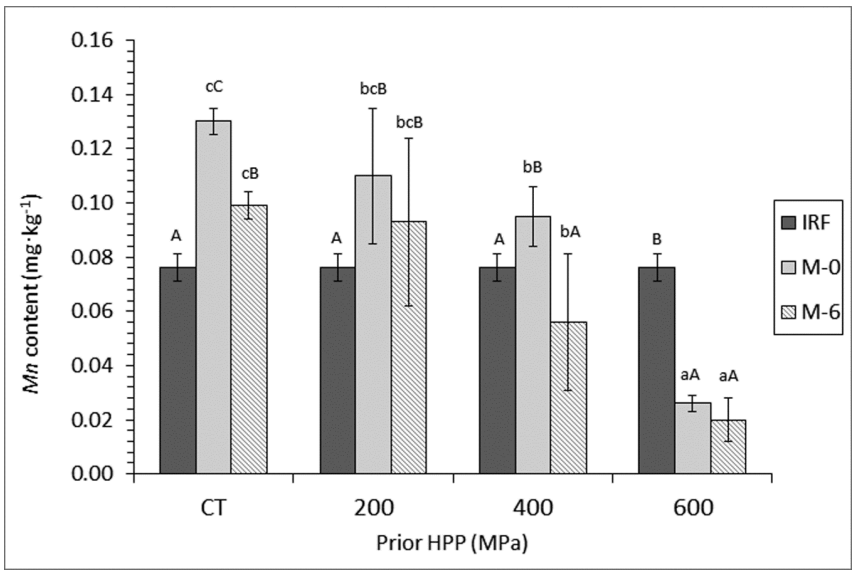

Figure 2. Determination of *Mn* content (mg·kg^{-1} dry muscle) * in initial raw fish (IRF) and canned mackerel subjected to high-pressure processing (HPP), freezing, and frozen storage **. * Mean values of three replicates ($n = 3$). Standard deviations are indicated by bars. For each frozen storage time (0 or 6 months), different lower-case letters (a–d) denote significant differences ($p < 0.05$) as a result of HPP. For each HPP condition, capital letters (A–C) denote significant differences ($p < 0.05$) as a result of frozen storage time. ** Sample name abbreviations as expressed in Figure 1.

Levels of *Fe* in canned fish showed a great general increase ($p < 0.05$) by comparing M-0 samples and initial raw fish (Table 3). However, a definite effect could not be implied ($p < 0.05$) when a subsequent frozen storage was encountered. Some differences were detected resulting from the prior HPP, although a definite trend could not be concluded ($p > 0.05$) for the presence of this essential element.

Table 3. Determination (mg·kg^{-1} dry muscle) of different transition metal elements (*Fe*, *Co*, and *Cu*) contents * in initial raw fish and canned mackerel subjected to different processing conditions **.

Element	Frozen Storage Time (Months)	High-Pressure Processing (HPP) (MPa)			
		CT	**200**	**400**	**600**
Fe	Initial raw fish	3.35 A (0.01)	3.35 A (0.01)	3.35 A (0.01)	3.35 A (0.01)
	0	7.51 cC (0.05)	6.64 bB (0.45)	7.18 bcB (0.35)	5.89 aB (0.11)
	6	6.48 aB (0.07)	7.28 bC (0.04)	6.51 abB (0.59)	6.86 abC (0.41)
Co	Initial raw fish	0.0018 A (0.0003)	0.0018 A (0.0003)	0.0018 A (0.0003)	0.0018 A (0.0003)
	0	0.0049 abB (0.0004)	0.0055 bcC (0.0007)	0.0042 aB (0.0004)	0.0065 cC (0.0006)
	6	0.0051 aB (0.0012)	0.0041 aB (0.0003)	0.0042 aB (0.0002)	0.0044 aB (0.0007)
Cu	Initial raw fish	0.36 A (0.03)	0.36 A (0.03)	0.36 A (0.03)	0.36 A (0.03)
	0	0.54 abB (0.05)	0.51 aB (0.04)	0.71 cB (0.07)	0.66 bcC (0.08)
	6	0.59 aB (0.06)	0.58 aB (0.05)	0.70 bB (0.02)	0.49 aB (0.05)

* Mean values of three replicates ($n = 3$). Standard deviations are indicated in brackets. ** In each row, different lower-case letters (a–c) denote significant differences ($p < 0.05$) as a result of HPP. In each column, capital letters (A–C) denote significant differences ($p < 0.05$) as a result of freezing and frozen storage.

The levels of *Co* and *Cu* in canned fish showed a general increase ($p < 0.05$) after HPP, freezing and canning (Table 3). However, the employment of a subsequent 6-month frozen storage led to scarce differences when compared with M-0 samples; thus, a remarkable content decrease ($p < 0.05$) was obtained in M-6 canned fish if previously subjected to a 600-MPa treatment. Concerning the effect of prior pressure treatment, a definite trend could not be implied in canned samples for both essential elements. For *Co*, higher average values were obtained in the 600-MPa batch when comparing the different kinds of batches corresponding to M-0 canned fish. Meantime, CT batch provided the highest average values if a prior 6-month frozen storage was also accomplished. In the case of *Cu*, higher average values were obtained in 400- and 600-MPa batches at the M-0 condition; for M-6 fish, *Cu* levels of 400-MPa batch were found higher ($p < 0.05$) than those corresponding to its counterpart batches.

Determination of *Cd* content in canned fish showed a general increase ($p < 0.05$) with processing when comparing the initial raw fish and samples corresponding to month-0 condition (Figure 3). However, comparison between M-0 and M-6 canned fish from the CT batch provided a marked *Cd* content decrease ($p < 0.05$) as a result of subjecting the fish to a frozen storage period. On the contrary, canned fish previously subjected to high-pressure did not provide differences ($p > 0.05$) as a result of the frozen storage period. Interestingly, a strong effect of prior pressure level applied was observed ($p < 0.05$). Thus, increasing contents for this toxic element were obtained with pressure level in samples corresponding to prior 0 and 6 months of frozen storage. Remarkably, higher *Cd* values were observed in 600- and 400-MPa batches when compared with their counterpart controls.

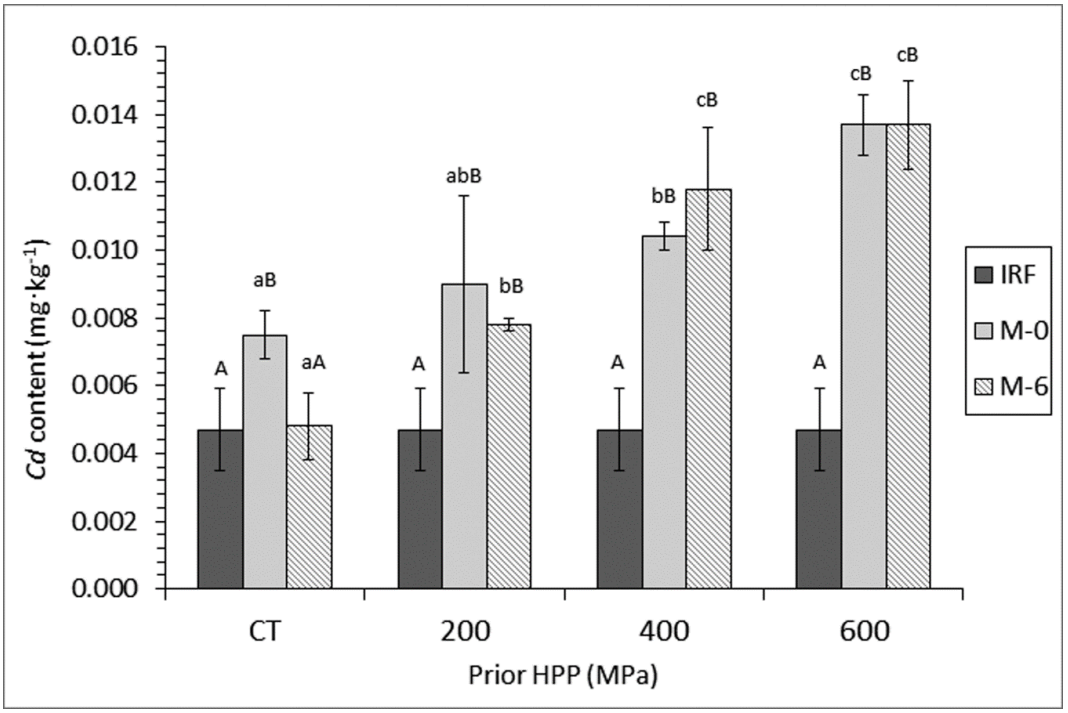

Figure 3. Determination of *Cd* content (mg·kg^{-1} dry muscle) * in initial raw fish (IRF) and canned mackerel subjected to high-pressure processing (HPP), freezing, and frozen storage **. * Mean values of three replicates (n = 3). Standard deviations are indicated by bars. For each frozen storage time (0 or 6 months), different lower-case letters (a–c) denote significant differences ($p < 0.05$) as a result of HPP. For each HPP condition, capital letters (A, B) denote significant differences ($p < 0.05$) as a result of frozen storage time. ** Sample name abbreviations as expressed in Figure 1.

3.3. Changes in the Content of Other Metals (Sn and Pb)

A general increase ($p < 0.05$) in *Sn* content was proved in canned samples corresponding to month-0 condition when compared with initial fish values (Table 4). If a frozen storage period is included as prior treatment to canning, an additional increase ($p < 0.05$) in *Sn* average levels in canned fish was observed in all batches. Differences were significant ($p < 0.05$) only for the CT batch. No significant effect ($p > 0.05$) of prior HPP was implied on the content of this toxic metal in canned fish; however, higher average values were observed in 200- and 400-MPa batches.

Table 4. Determination (mg·kg^{-1} dry muscle) of two metals (*Sn* and *Pb*) and two metalloid (*As* and *Se*) contents * in initial raw fish and canned mackerel subjected to different processing conditions **.

Element	Frozen Storage Time (Months)	High-Pressure Processing (HPP) (MPa)			
		CT	**200**	**400**	**600**
Sn	Initial raw fish	0.0011 A (0.0006)	0.0011 A (0.0006)	0.0011 A (0.0006)	0.0011 A (0.0006)
	0	0.0032 aB (0.0004)	0.0042 aB (0.0005)	0.0042 aB (0.0009)	0.0034 aB (0.0008)
	6	0.0045 aC (0.0002)	0.0048 aB (0.0003)	0.0053 aB (0.0005)	0.0047 aB (0.0005)

Table 4. *Cont.*

Element	Frozen Storage Time (Months)	High-Pressure Processing (HPP) (MPa)			
		CT	200	400	600
Pb	Initial raw fish	0.0127 B (0.0037)	0.0127 B (0.0037)	0.0127 B (0.0037)	0.0127 B (0.0037)
	0	0.0032 abA (0.0012)	0.0032 bA (0.0009)	0.0025 bA (0.0001)	0.0015 aA (0.0006)
	6	0.0040 aA (0.0013)	0.0037 aA (0.0000)	0.0039 aA (0.0015)	0.0030 aA (0.0008)
As	Initial raw fish	1.02 A (0.05)	1.02 A (0.05)	1.02 A (0.05)	1.02 A (0.05)
	0	1.20 aB (0.01)	1.47 bB (0.09)	1.30 abA (0.30)	1.16 aA (0.08)
	6	1.12 aAB (0.16)	1.15 aAB (0.09)	1.13 aA (0.10)	1.17 aA (0.10)
Se	Initial raw fish	0.42 A (0.02)	0.42 A (0.02)	0.42 A (0.02)	0.42 A (0.02)
	0	0.81 aC (0.07)	0.77 aB (0.06)	0.92 bC (0.01)	1.03 cC (0.04)
	6	0.62 aB (0.03)	0.74 bB (0.03)	0.76 bB (0.03)	0.78 bB (0.02)

* Mean values of three replicates ($n = 3$). Standard deviations are indicated in brackets. ** In each row, different lower-case letters (a–c) denote significant differences ($p < 0.05$) as a result of HPP. In each column, capital letters (A–C) denote significant differences ($p < 0.05$) as a result of freezing and frozen storage.

Determination of *Pb* content in canned fish revealed a strong general decrease ($p < 0.05$) as a result of HPP, freezing, and canning (Table 4). On the contrary, if a prior 6-month frozen storage is employed, higher average values were obtained in all batches if compared with canned samples corresponding to month-0 storage. However, such M-6 canned values were substantially lower ($p < 0.05$) than those corresponding to the initial fish. A decreasing effect of prior pressure-level applied could be outlined in M-0 samples, the lowest average values for this toxic element being obtained in the 600-MPa batch.

3.4. Changes in Metalloids (As and Se) Content

Average levels of *As* increased with HPP, freezing and canning in all kinds of samples; differences were found significant ($p < 0.05$) in the CT and 200-MPa batches (Table 4). However, if a 6-month frozen storage period is also included as pre-treatment, comparison with month-0 samples led to lower average values, but differences were not significant ($p > 0.05$). Concerning the effect of HPP, scarce differences were detected and a definite trend could not be signalled ($p > 0.05$) in values corresponding to this toxic element.

In all kinds of canned fish, higher *Se* levels were detected than in initial raw fish (Table 4). Nevertheless, comparison between M-0 and M-6 canned samples showed a relevant decrease as a result of including a 6-month frozen storage period; remarkably, this decrease was significant ($p < 0.05$) in CT, 400-, and 600-MPa batches. An average *Se* value increase was observed in canned fish by increasing the pressure level applied. Thus, lower values ($p < 0.05$) for this essential element were obtained in CT batch when compared with 400- and 600-MPa fish (M-0 samples) and when compared with all high-pressure treated batches (M-6 samples).

3.5. Changes in Non-Metal Elements (P and S) Content

A general decrease ($p < 0.05$) of *P* content was detected in canned samples corresponding to month-0 condition when compared with initial fish values (Table 5). Furthermore, subjecting the mackerel to a prior frozen storage period of 6 months led the *P* level in all batches to lower values

($p < 0.05$) than those obtained for M-0 fish. A decreasing effect with pressure level could also be outlined for the content of this macroelement; this effect was more remarkable in samples corresponding to M-0 condition. Thus, a lower ($p < 0.05$) value in month-0 canned fish corresponding to 600-MPa treatment was obtained when compared with their counterparts from CT and 200-MPa batches. For M-6 samples, canned fish related to 400- and 600-MPa batches revealed lower average values than their counterparts from CT and 200-MPa batches.

Table 5. Determination (mg·kg^{-1} dry muscle) of different non-metal elements (*P* and *S*) contents * in initial raw fish and canned mackerel subjected to different processing conditions **.

Element	Frozen Storage Time (Months)	High-Pressure Processing (HPP) (MPa)			
		CT	**200**	**400**	**600**
P	Initial raw fish	2.61 C (0.05)	2.61 C (0.05)	2.61 C (0.05)	2.61 C (0.05)
	0	1.95 cB (0.05)	1.86 bcB (0.15)	1.67 abB (0.13)	1.55 aB (0.03)
	6	1.32 aA (0.07)	1.35 aA (0.10)	1.26 aA (0.08)	1.23 aA (0.09)
S	Initial raw fish	2.72 A (0.06)	2.72 A (0.06)	2.72 A (0.06)	2.72 A (0.06)
	0	2.87 aB (0.06)	2.74 aA (0.11)	2.90 aB (0.07)	3.13 bB (0.09)
	6	2.74 aA (0.02)	2.68 aA (0.09)	2.73 aAB (0.10)	3.11 bB (0.06)

* Mean values of three replicates ($n = 3$). Standard deviations are indicated in brackets. ** In each row, different lower-case letters (a–c) denote significant differences ($p < 0.05$) as a result of the HPP. In each column, capital letters (A–C) denote significant differences ($p < 0.05$) as a result of freezing and frozen storage.

Related to *S* levels, average contents showed a general increase with processing when comparing initial values corresponding to raw fish and month-0 canned fish. Differences were significant ($p < 0.05$) in all batches except for 200-MPa fish (Table 5). Additionally, comparison between M-0 and M-6 canned samples provided a general decrease of average levels of this macroelement resulting from the frozen storage period. Differences were significant ($p < 0.05$) for the CT batch. Concerning the effect of prior pressure applied, a higher ($p < 0.05$) *S* content was detected in canned fish belonging to the highest pressure applied for both month-0 and month-6 conditions.

3.6. Changes of Moisture Content

Comparison between initial raw fish and canned fish showed no effect ($p > 0.05$) of HPP (200 and 400 MPa conditions), freezing, frozen storage, and canning on the moisture content (Table 6). However, if canned fish corresponding to the highest pressure condition is considered, a marked decrease of the moisture average value was obtained in canned fish, differences being significant ($p < 0.05$) if a 6-month storage period is also encountered as prior treatment. Furthermore, comparison among batches showed the lowest average values in fish corresponding to 600-MPa treatment both for M-0 and M-6 samples; differences with control were significant ($p < 0.05$) in fish canned after the freezing step and after a 6-month frozen storage.

Table 6. Moisture content ($g \cdot kg^{-1}$ muscle) * of initial raw fish and canned mackerel subjected to different processing conditions **.

Prior Frozen Storage Time (Months)	High-Pressure Processing (HPP) (MPa)			
	CT	**200**	**400**	**600**
Initial raw fish	717.2 AB (19.3)	717.2 A (19.3)	717.2 A (19.3)	717.2 B (19.3)
0	725.7 bB (4.1)	730.6 bA (19.2)	709.4 abA (18.5)	679.4 aAB (28.8)
6	707.8 bA (3.1)	699.3 abA (12.9)	709.1 bA (11.9)	678.4 aA (12.1)

* Mean values of three replicates ($n = 3$). Standard deviations are indicated in brackets. ** In each row, different lower-case letters (a, b) denote significant differences ($p < 0.05$) as a result of HPP. In each column, capital letters (A, B) denote significant differences ($p < 0.05$) as a result of freezing and frozen storage time.

4. Discussion

4.1. Changes in Moisture Content in Canned Fish

Differences between moisture values corresponding to raw fish and canned fish (Table 6) can be explained as a result of the different processing steps carried out. Thus, two basic and opposite effects can be pointed out. On one side, denaturation of muscle proteins during the different steps of processing (pressurisation, freezing, frozen storage, and canning), especially during the sterilisation step, would lead to a decrease of water-holding capacity, so that a substantial loss of water from the fish muscle should be produced [3,7,10]. On the other side, an interaction between the fish muscle and the hydrophilic brine-packaging medium ought to be produced [33,34]. Therefore, the fish muscle would be imbibed in the liquid medium, thus leading to a moisture content increase in canned muscle. Current results obtained indicate that most canned fish (CT, 200-, and 400-MPa batches) has not shown a significant effect ($p > 0.05$) on moisture level. Consequently, an equilibrium between both effects can be implied in such cases. On the contrary, samples corresponding to the highest pressure level batch revealed a marked decrease ($p < 0.05$) of moisture content, this showing an important effect of prior HPP on moisture content decrease in canned fish muscle.

Previous studies have shown that no changes in moisture content have been observed in canned fish muscle when packaged under an aqueous medium (i.e., brine) [34]. However, if an oily packaging medium was employed such as soya bean oil [35] or olive oil [34], a relevant decrease was detected in canned fish muscle. In a comparative study, brine and olive oil were used as packaging methods for canned freshwater nase (*Chondrostoma nasus*) [33]; it could be observed that both oil- and brine-packaged fish lost moisture, but this loss was higher in oil-canned fish as well as by increasing the *Fo* value applied.

4.2. Changes in Mineral Content in Canned Fish

Changes in mineral content (i.e., dry muscle basis) between the initial raw fish and canned samples have shown different trends (increase, decrease, or no variation) according to the element taken into account and the processing steps considered. In order to justify variation of mineral contents in the final canned fish, some revision of the most important events produced in each single processing step ought to be carried out.

Thus, HPP application on marine food has been focused on its ability for protein denaturation and consequently, the inhibition of endogenous and microbial deteriorative enzymes. The intensity and reversibility of this effect has shown to be strongly dependent on the strength of HPP conditions [9,10,13]. As a result of marked changes in protein structure by HPP, a water-holding capacity decrease is reported to occur, so that the resulting liquor loss may have an important detrimental effect on mineral retention in the fish muscle. According to data presented in Table 6, this effect would be expected to be especially important in the 600-MPa canned batch. On the other side, proteins in general, but the sarcoplasmic

fraction especially (around 25–30% of total proteins in fish), have shown to be especially labile to HPP, so that marked breakdown and content decrease have been proved in fatty [36] and lean [37] fish. As a consequence of this protein loss in the fish muscle, a relative content increase on other muscle constituents, such as minerals, would be expected to occur. Unfortunately, previous research related to the effect of HPP on mineral content in canned seafood and processed seafood in general is, to the best of our knowledge, not yet available.

Concerning the freezing and frozen storage of fish, different mechanisms of damage have been pointed out as being responsible for nutritional and sensory losses [5,7]. Among them, protein denaturation, microstructural changes, and lipid oxidation development have been signalled as most important for quality retention. As a result of freezing and frozen storage, the muscle becomes harder, more fibrous, less elastic, and losses its water-holding capacity [6,38]. As expressed for HPP, the resulting loss of water-holding capacity of proteins can be especially important for mineral content, since liquor produced from the muscle, especially during the required thawing step, can lead to important losses in mineral content. This mineral release would be expected to increase with time and temperature of frozen storage. However, previous research related to this loss can be considered scarce. Remarkably, one especially important consequence for quality retention would be the increase of the non-*Fe* content due to release of *Fe* from heme-*Fe* complexes by oxidative cleavage of the porphyrin ring [39,40].

Related to the effect of the canning process (namely, sterilisation step), and thermal treatments in general, marine species constituents have been revealed to be highly sensitive [2,41]. Among the most important events, heat breakdown and oxidation of constituents (namely, protein and lipid fractions), leaching of water-soluble constituents, and toughening and drying of fish muscle can be mentioned, all of them susceptible to exert an effect on mineral content [42,43]. Related to mineral content, and as for HPP and frozen storage, liquor losses from canned fish muscle may have a detrimental effect on mineral content, thus leading to substantial content decreases. Consequently, reduction in mineral contents of fish during the heating process may be related to the protein denaturation and release of these elements with the loss of water as free salts, possibly associated with soluble free amino acids, hydrophobic vitamins, and uncoagulated proteins [44–46]. This loss can be especially important when an aqueous medium is employed as a packaging medium. Remarkably, a higher fat content in the fish flesh produces lower losses of minerals, thus indicating a kind of interaction between both kinds of constituents [47,48]. On the other side, it is known that denatured proteins become more reactive and can be damaged easily by interacting with other constituents, especially if a strong process such as sterilisation is concerned. Furthermore, and as expressed above for frozen storage, release of prooxidant elements such as non-heme *Fe* from heme-*Fe* complexes may have important consequences on rancidity stability of fish muscle [39,49]. Thus, canned fish may undergo a substantial lipid oxidation development, thus leading to lipid breakdown and production of low-molecular-weight molecules susceptible to be lost from the canned fish muscle [3,34]. Therefore, a content decrease in constituents such as proteins and lipids in the canned fish muscle would lead to a relative increase of other constituents such as minerals.

Previous research has addressed the effect of canning on mineral content in fish muscle. Thus, some loss in minerals (*Na*, *K*, *Mg*, *P*, *Cu*, *Fe*, and *Ca*) from the muscle into the packaging medium was detected by Seet and Brown [50] in water-packaged canned tuna (*Thunnus alalunga*). Later on, Castrillón et al. [35] obtained a water and protein loss and fat content increase after albacore (*Thunnus alalunga*) canning (sterilisation at 115 °C for 55 or 90 min) by using soy bean oil as packaging medium; from raw to steamed fish, some elements content decreased (*Mg*, *K*, and *P*) and others remained the same (*Ca*, *Na*, *Zn*, *Cu*, and *Fe*). Ganjavi et al. [45] showed that defrosting, cooking, and sterilisation reduced the contents of *Pb* and *Cd* considerably in oil-canned yellowfin tuna (*Thunnus albacares*) and skipjack tuna (*Katsuwonus pelamis*) from the Persian Gulf and Oman Sea.

Consequently, and taking into account the different effects that each single processing step may have on the current canned mackerel, two opposite effects can be signalled related to changes in

the mineral content in the final canned muscle. One side, each of the different processing steps (HPP, freezing, frozen storage, and canning), especially sterilisation, would lead to denaturalisation, oxidation breakdown, and partial loss of the main constituents (proteins and lipids, especially) [7,10,41]. Therefore, a relative increase of other constituent content such as macrominerals and trace elements would be expected to occur. On the other side, modifications of the different constituents and consequently, breakdown of binding of minerals to other constituents, as well as liquor losses from the muscle would lead to a partial loss of minerals into the packaging medium. Among the different constituent modifications, protein denaturation can be of special significance for mineral content in fish muscle, as leading to a decreased water-holding capacity [10,45], and resulting in an increasing liquor loss susceptible to increase the mineral loss. The importance of this effect would depend on the kind of binding to other constituents of the fish muscle and the more or less hydrophilic/lipophilic nature of molecules they are integrated in [24,25]. Thus, elements whose linkage to other constituents is easily lost during any of the processing steps would lead to a decreased content. On the contrary, those whose linkage to other constituents is not modified during processing would not be likely to be lost and would increase their relative content in canned fish muscle according to the content decrease of other constituents such as proteins and lipids.

Consequently, mineral contents measured in the present study can be considered the result of these two opposite effects. Those elements that have shown to increase their content would imply a predominance of the first factor mentioned (i.e., partial loss of other constituents). On the contrary, a content decrease would signify that the second factor (i.e., liquor losses) has been more important. Furthermore, a balanced incidence of both factors would be produced when no substantial differences are found.

5. Conclusions

The content on different macroelements and trace elements in canned Atlantic Chub mackerel was analysed, taking into account the effect that prior HPP, freezing, frozen storage, and canning may exert. As a result, different trends were implied on the basis of changes in other constituents of the muscle (namely, protein denaturation, water loss, and lipid oxidation), liquor losses from the muscle, interaction of the fish muscle with the hydrophilic brine-packaging medium, the kind of binding of minerals to other constituents of the fish muscle and the more or less hydrophilic/lipophilic behaviour of molecules they are integrated in. It is concluded that elements that have shown to increase their content in canned muscle would imply a predominance of a partial loss of other constituents (i.e., protein and lipids fractions). On the contrary, a content decrease would signify that mineral losses from the muscle into liquor losses from the canned muscle resulting from protein denaturation would be more important. Furthermore, a balanced incidence of opposite factors would be produced when no substantial differences are found.

Thus, most elements (*Na*, *Ca*, *Ba*, *Mn*, *Fe*, *Cu*, *Cd*, *Sn*, *As*, *S*, and *Se*) showed an increased ($p < 0.05$) presence in mackerel muscle canned after freezing (M-0 samples). An additional content increase ($p < 0.05$) was observed by means of subjecting the mackerel muscle to a prior 6-month frozen storage for *Na* and *Sn* in canned fish, while other elements (*Ca*, *Ba*, *Mn*, *Fe*, *Cd*, *S*, and *Se*) showed decreased levels ($p < 0.05$) as a result of subjecting the fish to such a frozen storage period. On the contrary, prior freezing and canning led to lower values ($p < 0.05$) in canned fish for *K*, *Mg*, *Pb*, and *P*, such values being lowered ($p < 0.05$) if a prior 6-month frozen storage was also employed.

Concerning the effect of prior HPP, a relevant content decrease ($p < 0.05$) could be observed for *K*, *Mg*, *Ca*, *Ba*, *Mn*, *Fe*, *Pb*, and *P* in fish canned after the freezing step (namely, M-0 samples); additionally, a decrease ($p < 0.05$) was implied in *Ca*, *Ba*, and *Mn* values if a 6-month frozen storage period was undertaken (i.e., M-6 samples). On the contrary, prior HPP led to a marked increase ($p < 0.05$) with pressure level applied for *Cd*, *S*, and *Se* contents both in M-0 and M-6 canned samples. Finally, no effect ($p > 0.05$) of prior HPP was implied in canned mackerel for *Na*, *Sn*, and *As* levels. In most cases, HPP effect increased with pressure level.

Previous reports on mineral content in processed seafood can be considered scarce in general, being negligible in the case of high-pressure treated seafood. The current study represents a first approach on the knowledge of the effect of different pre-treatments on the content of macroelements and trace elements presence in canned fish. Further research ought to be addressed on the knowledge of the different biomolecules minerals are integrated on in order to justify the content increase/decrease resulting from processing. Additionally, and on the basis of the great impact of essential and toxic element levels in canned fish, further research ought to be addressed to analyse the effect of different pre-processing conditions such as prior frozen storage time, packaging medium employed for canning, and sterilisation condition (time and temperature).

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Iodine and Mercury Content in Raw, Boiled, Pan-Fried, and Oven-Baked Atlantic Cod (*Gadus morhua*)

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Abstract: There is a lack of scientific evidence regarding the stability of iodine and mercury during cooking and processing of seafood. In this study, the iodine and mercury content were determined after thawing frozen fillets of Atlantic cod (*Cadus morhua*), and further in raw compared to boiled, pan-fried, and oven baked fillets. Iodine was determined by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) and mercury by atomic absorption spectrophotometry with Direct Mercury Analyzer (DMA-80). Thawing of the cod resulted on average in a 12% loss of iodine to the thawing water. Boiling significantly decreased the total content of iodine per slice of cod fillet corresponding to the concentration of iodine found in the boiling water. Pan-frying and oven-baking did not cause any significant changes of the total iodine content per slice of cod fillet, although iodine content per 100 g increased due to weight reduction of the cod slices from evaporation of water during preparation. For mercury, we found minimal changes of the different cooking methods. In summary, the findings in our study show that boiling had the greatest effect on the iodine content in the cod fillets. Type of cooking method should be specified in food composition databases as this in turn may influence estimation of iodine intake.

Keywords: fish; cooking; processing; food composition data; analysis; ICP-MS; DMA-80; nutrition security

1. Introduction

Representative, local, up-to date high-quality food composition data are of fundamental importance for estimating nutrient intake in a population. Chemical analysis is the gold standard for generating data, but due to costs, analytical data comprise only a minor part of available information in most food composition databases (FCDB) and in food composition tables (FCT) [1]. Changes in nutrient content due to cooking method; for instance, boiling, frying, and grilling, and processing such as smoking, salting, and drying, should be considered when estimating nutrient intake of different foods [2]. Storage of fish by freezing is an often-used method to increase the shelf life of seafood. Thus, any changes of nutrient content during thawing, and further use of processing and cooking methods of the fish is important to quantify.

Presently, the Norwegian FCT [3] and the International Network of Food Data Systems (INFOODS) Food composition database (FCDB) [4] for fish and shellfish [5] only include analytical data on iodine in raw fish. In both the INFOODS FCDB [4] and the Norwegian FCT [3], values for cooked, fried, and processed fish are estimated or calculated, not chemically analyzed. Analytical data on

contaminants and heavy metals are not included in the Norwegian FCT, but such data in seafood are available in the open access Seafood database [6] at Institute of Marine Research (IMR). Atlantic cod (*Gadus morhua*) is the most commonly consumed lean white fish species in Norway, and total catch was 327,648 tons (live weight) in the Norwegian fisheries in 2019 [7]. Raw Atlantic cod and other lean fish species such as pollack (*Pollachius pollachius*), haddock (*Melanogrammus aeglefinus*), and saithe (*Pollachius virens*) have a considerably higher content of iodine than fatty fish species like mackerel (*Scomber scombrus*), herring (*Clupea harengus*), and farmed Atlantic salmon (*Salmo salar*) [8,9]. Iodine is an essential micronutrient for humans and has an important role in growth, brain development, and metabolism as it is active in the biosynthesis of thyroid hormones [10]. At the same time, the range for acceptable iodine intake for adults is from 150 to 600 µg/day and is considered relatively constricted compared to other micronutrients [11]. Thus, it is important to have high quality data on iodine in iodine rich foods such as Atlantic cod. However, Atlantic cod is also a source of mercury and methylmercury. This is of concern in relation to food safety, as humans are predominantly exposed to mercury through fish consumption [12]. The content of mercury in different fish species varies considerably and depends on factors such as type of species, geographical area, size, and age [13]. Knowledge about mercury exposure in humans is of importance as mercury in the form of methylmercury may have adverse effects such as impaired neurodevelopment of the fetus during pregnancy [14]. We have previously published analytical data on iodine and mercury in several raw lean fish species [13,15,16] and in processed fish products [17,18]. In the present study, the main aim was to analyze the total iodine and mercury content in Atlantic cod fillets after thawing and further after three different cooking methods, i.e., boiling, pan-frying, and oven-baking. To our knowledge, this is the first experiment presenting analytical data on iodine and mercury content in Atlantic cod during different cooking methods.

2. Materials and Methods

2.1. Fish Samples

The study included Atlantic cod (*Gadus morhua*) catched in the Barents Sea in October 2015 purchased from Lerøy Seafood Group ASA (bought after tender). The cod weight ranged from 1–2.5 kg and was immediately frozen in blocks of 25 kg, and stored as whole fish without the head and organs at −30 °C. In December 2015, the cod was thawed and fillet portions of approximately 200 g without skin were produced and frozen separately in strings of four fillets in each package (Bulandet Fiskeindustri AS, Lerøy Seafood Group ASA) before transported frozen to the IMR and stored in an outdoor freezing room at −30 °C pending analysis and delivery to participants in the randomized intervention study "Mommy food" [19].

2.2. Practical Procedure and Sample Preparation

The cod fillet packages were thawed overnight for approximately 18 h in a refrigerator at 4 °C before the experiment was performed in July 2017. In total, 30 cod fillets were used in the present study. Each fillet was divided into two portions of approximately 100 g each. Half of each cod portion of 100 g was kept raw (n = 30). The other half was further prepared for three different cooking methods either 1: boiled (n = 10), 2: pan-fried (n = 10), or 3: oven-baked (n = 10). All cod fillets were weighted before and after processing. Figure 1 shows a schematic overview of the sample preparation of the cod fillets.

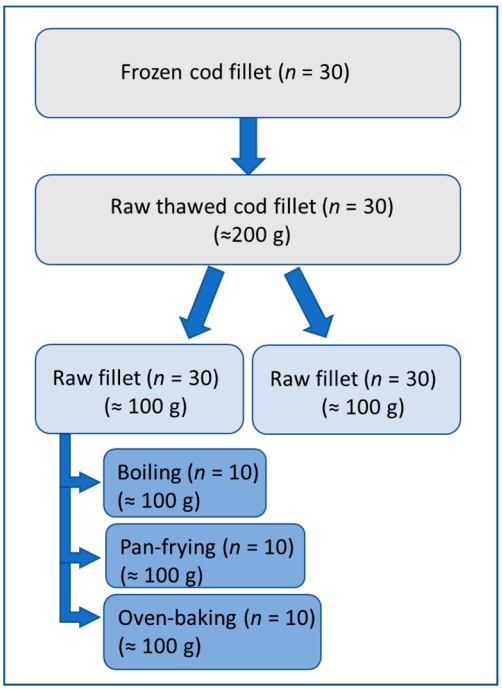

Figure 1. Schematic overview of cod fillet processing.

For cooking method 1, boiling, each cod fillet of approximately 100 g was put into a pan of boiling water and then stirred for 10 min in 1 L of water. For cooking method 2, pan-frying, the cod fillets were pan-fried for 6–7 minutes in 10 mL of rapeseed oil at medium temperature. For cooking method 3, oven-baking, the cod fillets were baked in the oven at 180 °C for 15 min. Thawing- and boiling water and any liquid left after pan-frying or oven baking were also collected for analyses of iodine. The experiment was performed using a household ceramic electric cooker (Gorenje, SuperPower Induction), a stainless-steel cooking pan (3 L of size), aluminum frying pan covered with Teflon (diameter 28 cm), and disposable aluminum form (0.5 L of size). No salt, spices, or food additives were used in the different cooking procedures.

After the experiment, all cod samples were freeze-dried (Labconco Freezone 18L Mod.775030, Kansas City, MI, USA) to constant weight using an accredited method according to ISO 17025. The samples were homogenized, and a sub-sample of the wet sample were weighed individually, put in separate plastic containers and frozen at minus 20 °C overnight. The samples were freeze-dried for 72 h (24 h at −50 °C, immediately followed by 48 h at +25 °C, with a vacuum of 0.2–0.01 mbar). The samples were then weighed once again, and the dry matter was calculated based on the difference in weight of the sample before and after freeze-drying. The method is validated, and measurement uncertainty is 10% for dry weight samples in the range of >10 to 99.5 g/100 g. Freeze-dried samples were then homogenized to a fine powder using a domestic mill and stored in twist off boxes at room temperature pending analysis. In total, 60 samples of cod (raw (n = 30) and processed (n = 30)) and 60 samples of liquid (i.e., thawing water (n = 30), boiling water (n = 10), liquid after pan-frying (n = 10), and oven baking (n = 10)) were collected. Iodine was determined in all samples (n = 120) and mercury was determined only in the cod fillet samples (n = 60), i.e., not in the liquid samples. To evaluate if the different cooking methods had any effect on iodine and mercury, we assessed the iodine and mercury content (µg) per piece of cod fillet and per 100 g (µg/100 g) cod fillet before and after cooking.

2.3. Determination of Iodine

For the determination of iodine, subsamples of ~0.2 g dry weight were added to 1 mL ultrapure tetrametylammonium hydroxide (TMAH) and 5 mL deionized water (>17 MΩ cm^{-1}, Nano pure-system, Nanopure, Barnstead, UK) before extraction at 90 °C ± 3 °C for 3 h. For the extraction of the liquid

samples, 4 mL of liquid was added to 1 mL TMAH. The liquid- and the cod samples were, after extraction, diluted to 10 mL and 25 mL, respectively with deionized water and left overnight for sedimentation of any solid particles. Aliquots of 10 mL were pipetted from the middle of the tubes in order to avoid taking up any precipitate from the bottom part of the solution. Prior to quantification, the samples were filtered through a 0.45 μm single use syringe and disposal filter. The 1% TMAH solution contained tellurium (1 mg/L) which was used as an internal standard in order to correct for instrument drift. Samples were analyzed against a standard addition calibration curve (2, 5, 10, 20, and 50 μg/L) to measure the unknown iodine content in the samples. Iodine was determined by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) with an iCap Q ICP-MS (Termo Fisher Scientific, Waltham, MA, USA) equipped with an autosampler (FAST SC-4Q DX, Elemental Scientific, Omaha, NE, USA). Limit of quantification (LOQ) is 0.32 μg/L or 0.04 mg/kg dry weight. Limit of detection (LOD) is 0.01 μg/L. The measurement uncertainty differs depending on the concentration range and is set to 15% for concentrations >10 × LOQ and 40% for concentrations between LOQ and 10 × LOQ. The trueness of the method was evaluated by analysis of Certified Reference Material (CRM). The CRM value for Fish muscle (BB 422) and skim milk powder (ERM-BD 150) is 1.4 ± 0.40 mg/kg and 1.73 ± 0.14 mg/kg for iodine, receptively. The trueness for iodine in CRMs used in the present study (n = 6) was in good agreement with the certified values and with the control chart of these two CRMs.

2.4. Determination of Mercury

For the determination of mercury, the cod samples were analyzed for total mercury by thermal decomposition, amalgamation, and atomic absorption spectrophotometry [20] using a Direct Mercury Analyzer (DMA-80, Milestone Srl, Italy). DMA-80 is calibrated in the linear area of mercury from 1.5–1000 ng. For samples in this area the accuracy is 80–120%. Samples were weighed on a calibrated four decimal scale from Sartorius (CP124S, Goettingen, Germany) and positioned in separated nickel boats prior to analyses. There are 40 positions for metal boats per analysis series in DMA-80. For each analysis series, there were empty metals boats at position 1 and 2 to make sure of no contamination from previous analyses. TORT-3: Lobster Hepatopancreas Reference Material for Trace Metals was used as CRM. A total of six samples of TORT-3 were placed at the beginning (n = 2), middle (n = 2), and end (n = 2) of the analysis series to check the accuracy of the method throughout the analysis. The CRM value for TORT-3 is 292 μg/kg for total mercury. Mean ± SD of analyzed CRM (n = 6) was 295 ± 11.4 μg/kg for total mercury, giving a mean accuracy of 101% (% relative SD: 3.9%). All results were within the accepted area of the analyses (±20%). Mercury determination was performed in two consecutive series and all analyzed values were above the LOQ of 0.08 ng mercury and the LOD of 0.02 ng.

2.5. Data Analysis and Statistical Methods

The descriptive statistics mean, standard deviation (SD) of mean, median, and range were conducted using Microsoft Office Excel 365 ProPlus (Microsoft Corporation, Redmond, WA, USA). For testing changes during cooking, percentage change from before to after cooking was used as this also normalized some of the variation from a few samples with high iodine levels. Statistica 13 (©Statsoft, Tulsa, OK, USA) was used to test if cooking methods had impact on the iodine and mercury content as well as dry weight percentages, using t-tests on percentage change, to test if these were different from zero.

3. Results

3.1. Total Iodine and Mercury Content in Raw Cod Slices after Thawing

The iodine and mercury content in thawed raw cod (n = 30) (~100 g/piece of cod fillet) and iodine in the associated thawing water are shown in Table 1, given as total μg per piece of cod fillets or in thawing water. The mean ± SD and median iodine content in the raw thawed cod fillets (n = 30) were

72 ± 87 µg and 52 µg wet weight, respectively. The iodine content (µg) ranged from 29.8 µg (cod #22) to 512.8 µg (cod #10) in the raw thawed cod fillets. Relative loss of iodine into the thawing water ranged from 5.5% (cod #23) to 19.9% (cod #21) with an average loss of 11.6 ± 3.4% (Table 1). The sample with the highest iodine content (cod #10) had similar loss into the thawing water compared to the other samples with lower iodine content. The mercury content in the raw thawed cod ranged from 1.3 µg (cod #19) to 6.3 µg (cod #13) per cod fillet wet weight with a mean ± SD and median mercury content of 2.6 ± 1.4 µg and 2.2 µg wet weight per cod sample (n = 30), respectively.

Table 1. Total iodine and mercury (µg) in raw cod fillets after thawing and iodine in thawing water (µg and %). Numbers are given as mean ± SD and (median) wet weight.

Sample	Iodine/Raw Fillet * (µg)	Iodine Thawing Water (µg)	Iodine Loss after Thawing (%)	Mercury/Raw Fillet (µg)
Cod fillet (#1–10)	101.4 ± 146.4 (53.0)	13.0 ± 19.6 (6.0)	12.3 ± 1.9 (12.2)	2.7 ± 1.3 (2.3)
Cod fillet (#11–20)	59.7 ± 32.7 (50.0)	7.2 ± 4.3 (6.7)	12.2 ± 3.1 (13.3)	3.0 ± 1.8 (2.4)
Cod fillet (#21–30)	54.7 ± 15.5 (53.4)	5.2 ± 1.8 (4.8)	10.2 ± 4.6 (8.9)	2.1 ± 0.8 (2.0)
All (n = 30)	71.9 ± 86.7 (52.0)	8.5 ± 11.7 (5.4)	11.6 ± 3.4 (11.7)	2.6 ± 1.4 (2.2)

* The other half of the cod fillets #1–10 were boiled, #11–20 were pan-fried, and #21–30 were oven-baked.

3.2. Dry Weight and Weight of the Cod Fillets before and after Different Cooking Methods

The dry weight (%) and weight (g) of the cod fillets before and after the different cooking methods are reported in Table 2. The mean ± SD dry weight in the cod fillets (n = 30) was 19.1 ± 0.6% before and 23.3 ± 2.0% after the different cooking methods. The dry weight increased significantly ($p < 0.01$), with the highest increase after pan-frying of the fillets.

Table 2. Dry weight (%) and weight (g) of the cod fillets before and after the different cooking methods and percent change of weight in wet weight. Numbers are given as mean ± SD and (median).

Cooking Method	Dry Weight before (%)	Dry Weight after (%)	Weight before (g)	Weight after (g)	Weight Change (%)
Boiling (n = 10)	18.9 ± 0.6 [a] (19.0)	21.0 ± 0.7 [b] (20.7)	99.6 ± 7.4 [a] (99.8)	84.2 ± 7.5 [b] (84.5)	15.5 ± 3.5 (16.6)
Pan-frying (n = 10)	19.0 ± 0.5 [a] (19.0)	25.6 ± 0.9 [b] (25.7)	100.7 ± 7.1 [a] (100.8)	82.1 ± 7.4 [b] (81.6)	18.6 ± 1.9 (18.0)
Oven-baking (n = 10)	19.5 ± 0.6 [a] (19.4)	23.2 ± 0.5 [b] (23.2)	103.6 ± 7.3 [a] (100.2)	82.5 ± 7.4 [b] (81.8)	20.4 ± 2.4 (20.0)
All (n = 30)	19.1 ± 0.6 (19.0)	23.3 ± 2.0 (23.2)	101.3 ± 7.2 (101.2)	82.9 ± 7.2 (20.0)	18.2 ± 3.3 (18.6)

Different letters denote significantly differences between dry weight rows and between weight rows ($p < 0.01$).

The weight of the raw cod fillet (n = 30) varied from 77.2 g (cod #11) to 110.1 g (cod #23) with a mean ± SD weight of 101.3 ± 7.2 g. The weight decreased significantly ($p < 0.01$) in all fillets after the different cooking methods and the weight loss ranged from 9.1% to 24.3% with a mean ± SD and median of 18.2 ± 3.3% and 18.6%, respectively.

3.3. Iodine and Mercury Content per Cod Fillet and Content per 100 g before and after Different Cooking Methods

The iodine content per fillet (µg) and per 100 g fillet (µg/100 g) before and after boiling, pan-frying, and oven-baking the cod fillets are shown in Table 3. Boiling the cod fillets (n = 10) in one liter of water reduced the iodine content per fillet with approximately 20%, but was not significant. The mean ± SD iodine concentration in the boiling water was 32 ± 43 µg and corresponded to an approximately

30% loss of iodine to the boiling water. The iodine content given as per 100 g fillet was significantly decreased by approximately 10%. Pan-frying and oven-baking did not cause any significant changes to the total iodine per fillet, although iodine content per 100 g increased due to weight reduction of the cod slices from evaporation of water during preparation. Figure 2 shows the percentage change of iodine given as total iodine content per fillet (µg) and as iodine content per 100 g (µg/100 g) before and after the different cooking methods.

Table 3. Total iodine (µg) per piece of cod fillets and in the associated liquid (µg), and iodine content (µg/100 g) in cod fillets before and after the different cooking methods. Numbers are given as mean ± SD and (median) wet weight.

Cooking Method	Iodine/Fillet, before * (µg)	Iodine/Fillet, after ** (µg)	Iodine Liquid *** (µg)	Iodine Content before (µg/100 g)	Iodine Content after (µg/100 g)
Boiling (n = 10)	114.6 ± 175.1 [a] (55.3)	79.4 ± 101.9 [b] (40.5)	32.5 ± 43.4 (14.8)	116.9 ± 182 [a] (56)	97.6 ± 130.8 [a] (44.5)
Pan-frying (n = 10)	65.9 ± 29.1 (56.2)	63.9 ± 30.4 (49.5)	1.3 ± 0.5 (1.2)	66.7 ± 33.8 [a] (57)	79.5 ± 43.9 [b] (62.5)
Oven-baking (n = 10)	61.6 ± 18.1 (62.4)	55.7 ± 15.4 (57)	9.3 ± 3.4 (16.6)	59.1 ± 15.6 [a] (60.5)	67.2 ± 16.4 [b] (70)
All (n = 30)	80.7 ± 102.4 (57.6)	66.3 ± 60.7 (49.4)	14.4 ± 27.8 (9.6)	80.9 ± 106.7 (56.5)	81.4 ± 78.5 (62.5)

* Iodine was calculated by multiplying the analyzed iodine concentration in the raw cod slice with the weight of the cod slice before cooking. ** Iodine was calculated by multiplying the analyzed iodine concentration after cooking with the weight of the cod slice after cooking. Different letters denote significant differences between rows $p = 0.001$. *** Iodine in liquid (%) was calculated by dividing analyzed iodine concentration in the liquid with analyzed iodine content in the raw cod slice and then dividing by 100. Different letters denote significant differences between iodine per fillet rows and between iodine content per 100 g fillet rows ($p < 0.01$).

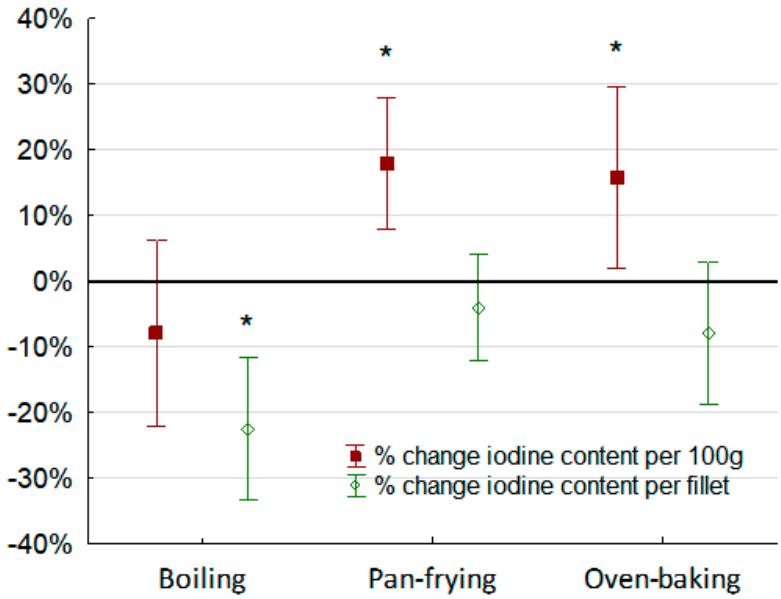

Figure 2. Percentage (%) change of iodine before and after different cooking methods (wet weight). Red bars show change in iodine content (µg/100 g) and green bars show change in total iodine per cod fillet (µg). Significant differences within cooking method are indicated by * ($p < 0.01$).

The mercury content before and after boiled (n = 10), pan-fried (n = 10), and oven-baked (n = 10) cod samples are shown in Table 4. The mean ± SD mercury per cod fillet in all cod samples (n = 30) was 2.8 ± 1.5 µg before and 2.8 ± 157 µg wet weight after the different cooking methods and were not significantly different from each other ($p > 0.05$). The mercury content per 100 g fillet (µg/100 g) increased after the three different cooking methods using wet weight. Since mercury will not dissolve

in the water phase, we used the dry weight data and found no significant ($p > 0.05$) differences between the different cooking methods.

Table 4. Total mercury per fillet (μg) and mercury content per 100 g fillet (μg/100 g) in cod fillets before and after the different cooking methods. Numbers are given as mean ± SD and (median) wet weight.

Cooking Method	Mercury/Fillet, before (μg)	Mercury/Fillet, after (μg)	Mercury Content before (μg/100 g)	Mercury Content after (μg/100 g)
Boiling (n = 10)	2.9 ± 1.6 (2.5)	2.7 ± 1.4 (2.4)	2.9 ± 1.3 (2.5)	3.2 ± 1.5 (2.8)
Pan-frying (n = 10)	3.3 ± 1.9 (2.7)	3.5 ± 1.9 (3.1)	3.3 ± 1.8 (2.7)	4.3 ± 2.2 (3.6)
Oven-baking (n = 10)	2.4 ± 0.8 (2.1)	2.2 ± 0.8 (2.0)	2.3 ± 0.8 (2.1)	2.7 ± 1.0 (2.5)
All (n = 30)	2.9 ± 1.5 (2.4)	2.8 ± 1.5 (2.3)	2.8 ± 1.4 (2.4)	3.4 ± 1.7 (2.8)

4. Discussion

The present study has assessed the effect of thawing, boiling, pan-frying, and oven-baking on the content of iodine and mercury in Atlantic cod fillets. In general, thawing of frozen cod fillets caused an approximately 12% loss of iodine to the thawing water. Boiling significantly decreased the total iodine per piece of cod fillet and the corresponding amount of iodine was found in the boiling water. Pan-frying and oven-baking did not cause any significant changes in the iodine per fillet, although iodine content per 100 g increased due to weight loss of the slices from evaporation of water during cooking. For mercury, we found minimal changes of the different cooking methods.

We found that boiling of the cod fillets significantly decreased the total content of iodine per piece of cod and that this loss was almost equal to the iodine concentration found in the boiling water. Thus, the average iodine loss in μg in the cod fillets can be explained by loss to the boiling water. The iodine content per 100 g fillet was reduced due to increased dry weight and reduced weight of the cod fillet after boiling. Steaming (105 °C in aluminum foil for 15 min) has previously been shown to not affect iodine content in hake (*Merlucius australis*), monkfish (*Lophius piscatorius*), mackerel (*Scomber scombrus*), tuna (*Katsuonus pelamis*), plaice (*Pleuronectes platessa*), mussel (*Mytilus edulis*), octopus (*Octopus vulgaris*), and shrimp (*Litopenaeus vannamel*) [21]. In the study by Doh et al., 2019, they found more than 60% loss of iodine after boiling abalone (*Haliotis discus hannai*) and 32% reduction after steaming or grilling [22]. Some of the explanation for higher loss of iodine from abalone may be that they are invertebrates, with open circulatory system, which may lead to greater loss of water-soluble components during boiling. Invertebrates also have higher concentrations of osmolytes in the tissues as they are osmoconformers and this may further contribute to greater loss of iodine. Although the loss is different between our study and Doh et al., 2019, it is reasonable that some iodine will be lost during boiling as iodide is reactive with the potential to undergo oxidation and reduction reactions within the food matrix [22]. Even if the iodine loss after boiling was higher per fillet than per 100 g cod fillet in our study, our results indicate a reduction of iodine in the range of 10–20%.

Most studies have primarily assessed the fate of iodized salt in a variety of foods after different cooking methods [23–26], and there are few studies investigating the effect different cooking- and processing methods may have on iodine content in fish and other seafood. In the study by Longvah et al., 2013, they reported an average loss of 47% iodine in different recipes from the Indian kitchen using iodized salt after boiling and the range of loss was from 14 to 88% [24]. The same study also reported a minor loss of iodine with cooking methods such as steaming, deep frying, and pressure cooking of the different recipes. Loss of iodine from the use of added iodized salt in food/recipes is not exactly comparable to the aim in the present study, but will be relevant when estimating iodine intake from the diet.

Pan-frying and oven-baking of the cod fillets showed minor changes of the total content of iodine (μg) per piece of cod in the present study. As a result that the dry weight percentage increased and the weight of the cod fillets decreased due to evaporation of water, we observed that the iodine content per 100 g (μg/100 g) in wet weight increased with 15–20% after pan frying or oven baking. We therefore assume that these two cooking methods are better in regard to preserving the iodine content compared to boiling. However, if cod is part of a dish where also the water is consumed (e.g., soup), the iodine loss will be less and comparable to pan-frying and oven-baking.

Different studies investigating mercury in seafood shows that cooking in general tends to increase the wet weight content of mercury in seafood, most likely due to loss of moisture during the cooking process [12,27,28]. Our results support these findings regarding mercury since we also observed a minor decrease of the moisture after pan-frying and oven-baking. In a study with Spanish mackerel (*Scomberomorus maculatus*), cat shark (*Scyliorhinus sp.*), and red tuna (*Thunus* thynnus), the dry weight mercury content for all these three fish species were slightly higher after boiling compared to frying and raw fish [29]. In the same study, they found that boiling and frying reduced mercury bioaccessibility by 40% and 60%, respectively, compared to raw fish mercury bioaccessibility. Bioaccessibility is the proportion of the mercury that potentially reaches the systemic circulation. The mercury bioaccessibility ranged from 10% in octopus (*Octopus vulgaris*) to 60% in monkfish (*Lophius piscatorius*) [21]. Thus, although the mercury after different cooking methods is almost unchanged in the present study, the estimation of exposure of mercury can probably be overestimated due to a reduced bioaccessibility of the mercury after cooking [21]. However, this was not assessed in this experiment and must be explored further.

As frozen cod were used in the present study, we were able to study if there were any changes of iodine content during thawing. The cod was frozen and thawed twice before we performed the different cooking methods of the portion packed cod. Given an average 12% loss of iodine due to thawing, the fresh caught cod may therefore originally have had up to a 25% higher iodine concentration. Using this approach, the iodine content in the fresh caught cod was approximately 100 μg/100 g fillet. However, this finding has probably no important relevance for estimating iodine intake from cod, since cod should not be eaten raw due to parasites.

The large variation of iodine between and within fish species, but also in relation to condition factor ($100 \times \text{weight/lenght}^3$), season, and geographical location [9,15], is a challenge when estimating the iodine intake from cod. The reported range of iodine in raw Atlantic cod fillets was 22 to 720 μg/100 g (n = 121) in the paper by Nerhus et al., 2018 and 18 to 1270 μg/100 g (n = 125) in the paper by Julshamn et al., 2001 [8]. Atlantic cod is regarded as a fish species relatively low in mercury; however, there are also large intraspecies variations. Still, the mercury content in cod varies less compared with iodine and ranged from 1 to 54 μg/100g (n = 516) with a mean of 11 ± 7 μg/100 g in a study from the North Sea and costal Norwegian waters and from 1 to 16 μg/100g (n = 804) with a mean of 3.6 ± 2.3 μg/100 g in samples from the Barents Sea [15]. The mercury content in the present study was approximately 3 μg/100 g raw fillet.

In the Norwegian FCT [3] boiled (fillet), oven-baked, and pan-fried are given the same value as raw cod (279 μg/100 g), while sliced cod has lower value (194 μg/100 g). In the INFOODS FCDB, boiled and grilled cod are given values higher than raw as seen in the present study as well, ranging from 280–400 μg/100 g. In both the INFOODS FCDB [4] and the Norwegian FCT [3], these values are estimated. Our study contributes with novel and new data on the effect of different cooking methods on the iodine level that further can be implemented in FCDBs and FCTs. With the shortage of studies regarding the effect of the cooking process on iodine, we can only speculate and assume that loss of iodine after boiling is most likely the same for other lean fish species.

Representative and reliable analytical food composition data are considered essential for estimating and evaluating the nutrient intake of individuals and population groups. To estimate intake of iodine and mercury exposure, representative and reliable analytical data are essential. Therefore, our study will provide new insights and reliable information that will increase the quality of iodine values after

different cooking methods of cod fillets in FCDBs and FCTs, and further the quality of data reporting the dietary iodine intake. FCDBs and FTCs would also benefit in including the most important food safety parameters like e.g., mercury. A limitation of our study is the relatively low number of samples for each cooking method and the smaller variation in the iodine content compared to other studies reporting iodine content.

5. Conclusions

The present study has determined the effect of thawing and different cooking methods on the content of iodine and mercury in Atlantic cod. Boiling decreased the iodine content per fillet and per 100 g fillet with approximately 10–20%. Pan-frying and oven-baking caused minor changes in the net iodine content, while content per 100 g increased due to reduced moisture. For mercury, we found minimal changes of the different cooking methods. Further studies are warranted to better understand the variation in iodine content in different fish species fillets and type of processing should be specified in food composition databases.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

CRM	Certified Reference Material
DMA-80	Direct Mercury Analyzer
FCDB	Food Composition Databases
FCT	Food Composition Tables
ICP-MS	Inductively Coupled Plasma-Mass Spectrometry
IMR	Institute of Marine Research
INFOODS	International Network of Food Data Systems
LOD	Limit of Detection
LOQ	Limit of Quantification
TMAH	Tetrametylammounium Hydroxid

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