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ORIGINAL PAPER

Identification of iron in foods: Mössbauer spectroscopy study

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Abstract

Iron is a multifunctional trace element that plays a key role in the growth and development of the human body at all stages of its life. This element is a nutrient that the human body cannot synthesize and must be obtained through food. Iron deficiency develops when the amount of iron in the diet is insufficient, leading to anemia and other pathological changes in the body. Iron is an essential ingredient in many food products, and with a measure of willingness, it is possible to ensure an adequate intake of this element. Limited research has examined the mineral content of plant or animal-based foods, especially iron. For this reason, this paper used Mössbauer spectroscopy and X-ray fluorescence methods to examine the concentration and properties of iron in foods. The results of studies using Mössbauer spectroscopy present the chemical states of iron naturally occurring in food products. Such studies have not been conducted before. The content of other essential micro- and macronutrients for our body, such as zinc, potassium, magnesium, and calcium, was also determined. Such knowledge is particularly relevant in the current environment, with an increasing number of people adopting a plant-based diet. Our research is based on products readily available in supermarkets or butcher shops. Among others, we used products such as black olives, soybeans, pumpkin seeds, beef, pork, and poultry liver. The research shows that just 100 grams of animal products provide an adequate daily dose of iron. The iron in these products is primarily heme iron, with only beef spleen, pork spleen, and pork liver containing nonheme iron. The plant-based products studied should not be considered a sufficient source of this element in the daily diet. However, these products contain significantly more magnesium and calcium than animal products.

Keywords: dietary iron, iron content, heme and nonheme iron, animal products, plant products, Mössbauer spectroscopy

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INTRODUCTION

The elements of which the human body is composed can be divided into three groups: bulk elements, macro-minerals, and trace elements. First, there are the bulk elements – carbon (C), oxygen (O), hydrogen (H), and nitrogen (N) – that make up about 96% of the human body (Zoroddu et al. 2019). These four elements are fundamental to the basic structure of all biochemical molecules, including lipids, proteins, and carbohydrates (Samanth 2024). Macro-minerals (macro-elements) include calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na), potassium (K), and chloride (Cl) – Zoroddu et al. (2019), Adeeb Hussein Ali (2023), Samanth (2024). These minerals are essential for nutrition. The remaining 0.15 % of the human body composition is made up of trace elements, namely manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), selenium (Se), cobalt (Co), molybdenum (Mo), and iodine (I) – Zoroddu et al. (2019), Samanth (2024). Trace elements are crucial for biological, chemical, and molecular processes that occur in cells. They serve, among other functions, as stabilizing centers for the structure of proteins and enzymes. These elements are therefore crucial for maintaining human health. Importantly, they must be obtained from food, as the human body cannot produce them (Samanth 2024). The most abundant trace element in the human body is iron. This element is also a biologically essential component of all living organisms. Iron occurs in the body primarily in complex forms, bound to proteins (hemoproteins), such as heme compounds (hemoglobin or myoglobin), heme enzymes (cytochromes), or nonheme compounds (ferritin). Furthermore, Fe can bind with sulfur (Fe-S), forming proteins with redox properties (Zoroddu et al. 2019, Grijota et al. 2022, Huenchuguala, Segura-Aguilar, 2023). Two types of iron can be found in foods, namely heme and nonheme. Heme iron is derived from hemoglobin and nonheme iron is extracted from all the other compounds present in food (Kongkachuichai et al. 2002). Although plant materials contain only nonheme iron, animal products contain both heme and nonheme iron. Nonheme dietary Fe is mainly in the ferric form (Fe^{3+}), which is not bioavailable and must be reduced to the ferrous form (Fe^{2+}) – Grijota et al. (2022). Heme Fe has greater bioavailability (15-35%) than nonheme Fe (2-20%) – Wheal et al. (2023). What is more, absorption from vegetables or cereal foods is usually less than 5% as compared with 15-20% absorption from animal sources such as beef, liver, or fish (Cook, Monsen 1976, Piskin et al. 2022). The average person's body needs 1-2 mg (Correnti et al. 2024, Samanth 2024) of iron per day to compensate for non-specific iron losses. Taking into account the absorption of iron from food, we need to supply it in the amount of 20-30 mg. Both iron deficiency and iron overload may lead to heart damage, anemia, and other pathological changes in the body (Ellwange et al. 2022, Sochanowicz et al. 2022).

Food should provide the human body with all the necessary nutrients, vitamins, and macro- and microelements for optimal growth and development, as well as for maintaining the body's internal balance. Therefore, analyzing the content of essential elements in food can help pinpoint possible causes of symptoms and illnesses. Such knowledge is also crucial for developing a well-balanced diet to prevent infections resulting from a lack or excess of specific elements in the body. An essential element is a chemical element necessary for an organism's survival or normal functioning since it plays a vital role in the body's metabolism. One of these more critical elements is iron. This element is commonly considered a mineral of concern for deficiency in people following vegetarian and vegan diets (Eberl et al. 2022). Such diets are associated with an increased consumption of phytate-containing vegetables, legumes, and grains, which hinder iron bioavailability. In this paper, we present results demonstrating the content of essential elements, particularly iron, which is crucial for the body's proper functioning. Both animal and plant products, considered the richest sources of these elements, particularly Fe, were selected for analysis. X-ray fluorescence analysis was used to determine the elemental content of the investigated products. Mössbauer spectroscopy is a powerful tool for studying the electronic structure, magnetic properties, molecular symmetry, and bonding properties of many materials. The results of this technique allow us to characterize the chemical states of iron naturally occurring in food products. Such data have not been previously published, yet it can provide valuable information not only in the context of proper nutrition, but also concerning the form and role of iron in both animal and plant organisms. Furthermore, analysis of the content and proportions of individual minerals will show possible causes of the symptoms and diseases. These elements are typically required in relatively small amounts and are usually obtained through diet.

MATERIALS AND METHODS

Animal products considered the richest in iron were selected for the study: beef, pork, poultry liver, beef and pork spleen, and pork heart. These products are readily available in supermarkets and butcher shops. Approximately one kilogram of animal products was selected and purchased. Since we typically do not consume them raw, they were cut into small pieces, dried at approximately 70°C for 20 min, and then ground into powder. Beef and pork blood were also used in the study. This blood is readily available and often used in the meat industry as a blood protein in products such as sausages, black pudding, luncheon meat, and ham. Dried blood is sold in packages ranging from 50 g to 5 kg. The product used in this study was sourced from the Domowa Spiżarnia store in Kielce, Poland. The plant products used in the study included pumpkin seeds, soybeans, olives, and sesame seeds. Randomly selected packages of these products were purchased from a store. These products were only ground in a mortar. Additionally, spirulina (a blue-green microalga classified as a cyanobacterium) and chlorella (single-celled green algae), plant-derived products recommended for vegetarians and vegans as a rich source of iron, were also used in the study. Chlorella comes from South Korea (distributor: FoodWell Sp. z o.o., Warsaw, Poland). Each package contains 21 g of product, equivalent to 7 daily servings. Spirulina packages (manufactured by A-Z Medica Sp. z o.o., Gdańsk, Poland) contain approximately 41 g of product, equivalent to approximately 10 daily servings. Figure 1 shows selected food products prepared for measurements.

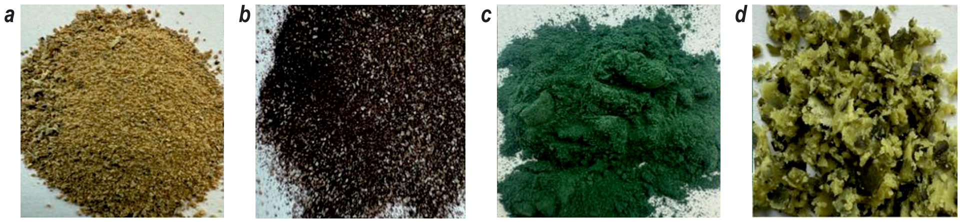

Fig 1. Selected food products prepared for measurements: dried and powdered pork liver (*a*), dried pork blood (*b*), spirulina (*c*), and pumpkin seeds ground in a mortar (*d*)

The chemical composition of the initial siderite samples was determined by X-ray fluorescence (XRF) with a ZSX Primus II Rigaku spectrometer. The spectrometer, equipped with a 4 kW, 60 kV Rh anode and wavelength dispersion detection system, allowed for the analysis of the elements from Be to U. No external standards were necessary as the Fundamental Parameters (FP) method was used. The XRF FP method is a "standardless" theoretical approach to quantitative analysis. Instead of relying on physical calibration standards, it calculates elemental concentrations directly from measured X-ray intensities using a database of fundamental physical constants and a precise model of the spectrometer. Sample preparation for XRF elemental analysis requires a smooth and uniform sample surface. Therefore, the powdered samples were pressed in a specialized laboratory hydraulic press to obtain tablets. According to the manufacturer, the global uncertainty in determining the content of individual elements is about 1%. However, the uncertainties may be about ~15% for trace elements (Rousseau 2001).

The ^{57}Fe Mössbauer transmission spectra were recorded at room and high temperatures using an Integrated Mössbauer Spectroscopy Measurement System (designed by Wacław Musiał and Jacek Marzec, Poland), and a source ^{57}Co:Rh (25 mCi). A gas proportional counter LND-45431 was used as a gamma-ray detector. The spectrometer velocity scale was calibrated at room temperature with a 25 μm thick α-Fe foil. Data were collected with a 1024-channel analyzer (before folding). All Mössbauer measurements were carried out on powdered samples. The Mössbauer spectra were evaluated by least-square fitting of the lines using the MossWinn 4.0i program. The spectral parameters, including isomer shift (IS), quadrupole splitting (QS), full line width at half maximum (G), and relative subspectrum area (A), were determined. Some spectra were fitted with a quadrupole splitting distribution (QSD). The model and implementation are based on the Voigt-based fitting method (Rancourt, Ping 1991). The isomer shifts are given relative to α-Fe at room temperature.

RESULTS AND DISCUSSION

The content of bulk and macro elements for all the food products investigated is presented in Figure 2. In animal products, the main elements are oxygen, nitrogen, and carbon (Figure 2*a*). Hydrogen is not included here because the method used does not allow for its measurement. These elements,

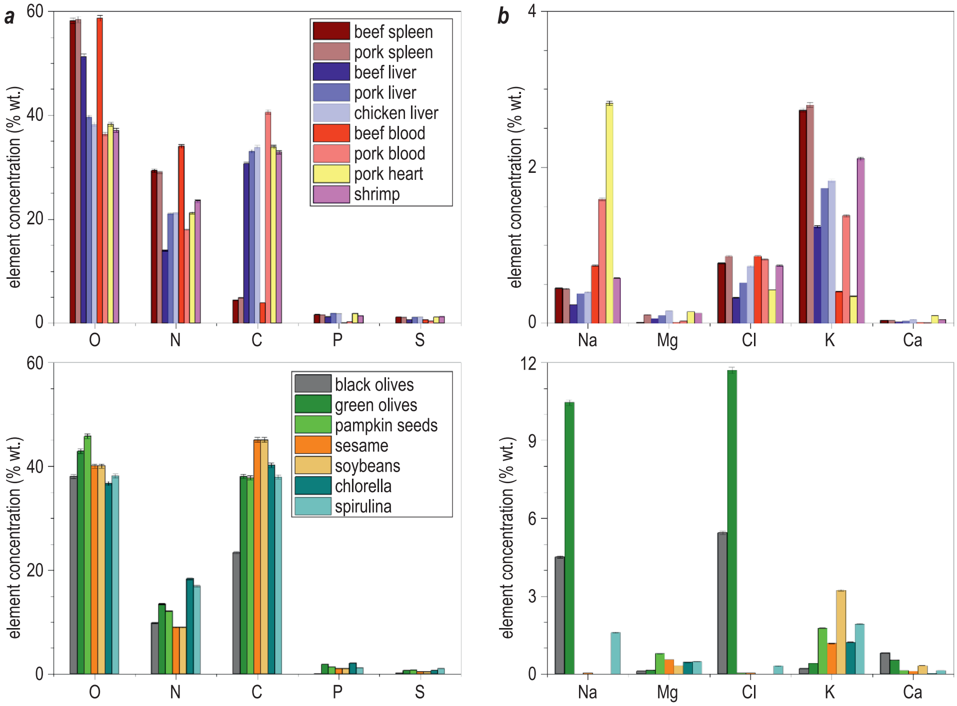

Fig. 2. Content of (*a*) biogenic and (*b*) macro elements in the investigated products of plant and animal origin based on the results of X-ray fluorescence analysis (XRF). The uncertainties of individual elements are marked on the graphs

along with phosphorus and sulfur, are biogenic elements that are essential for life because the body uses them to build structures and consumes them in metabolic processes. In addition to P and S, other macroelements, such as sodium, potassium, calcium, chromium, and magnesium, are also present in these samples (Figure 2*b*). Plant foods contain mainly C, N, and O, which are the main components of organic matter. Plant products contain slightly more macro elements, such as Mg and Ca, than animal products (Figure 2*b*).

It is also worth noting that olives contain significant amounts of sodium and chloride. The content of these two elements is at least ten times higher than in other products (Figure 2*b*). Raw olives are bitter and are soaked in brine to make them edible, hence the high content of sodium and chloride in olives. For this reason, people with hypertension or those on a low-sodium diet should limit their olive consumption to just a few per day. It is worth noting that five olives weigh about 15 grams.

All samples also contained iron, one of the trace elements, and samples of animal origin additionally contained zinc. We remember that these are essential elements necessary for the proper functioning of the body, which it does not produce and which must be obtained through food. Table 1 provides information on the content of these elements in 100 g of the product and the recommended daily allowance (RDA) for these elements in adult men, which is 12 mg for Fe and 9.4 mg for Zn (Kennedy, Meyers 2005, Ma et al. 2021, Yoon, Choi 2023). It is worth noting that the recommended daily allowance (RDA) for women is slightly higher for Fe and lower for Zn (Kennedy, Meyers 2005, Ma et al. 2021, Yoon, Choi 2023). The weight of 100 g of the product was chosen because this is the amount of meat an adult should consume daily. Also, most food products contain information about the vitamin and mineral content per 100 grams of the product. Additionally, Table 1

Table 1

Content of selected elements in 100 g of investigated food products, calculated based on the results of the X-ray fluorescence method, and their recommended daily allowance (RDA). All values are given with an accuracy of ~15%3

Common name	Fe (mg)	Fe_{RDA} (%)	Zn (mg)	Zn_{RDA} (%)	Mg (mg)	Mg_{RDA} (%)	Ca (mg)	Ca_{RDA} (%)
Beef spleen	920	7667	150	1596	100	25	40	3
Beef blood	430	3583	10	106	10	3	10	1
Beef liver	50	417	10	106	60	15	20	2
Pork spleen	180	150	180	1915	110	28	40	3
Pork blood	310	2583	20	213	30	8	10	1
Pork liver	160	1333	70	745	100	25	30	2
Chicken liver	120	1000	190	2021	160	40	50	4
Pork heart	100	833	30	319	150	38	350	27
Shrimp	50	417	70	745	130	33	50	4
Black olives	130	1083	-	-	120	30	810	62
Green olives	10(2)	83	-	-	150	38	540	42
Pumpkin seeds	30	250	-	-	790	198	140	11
Sesame	20	167	-	-	560	140	120	9
Soy seeds	20	167	-	-	320	80	330	25
Chlorella	50	417	-	-	460	115	30	2
Spirulina	80	667	-	-	480	120	130	10

provides the content and RDA for elements such as magnesium and calcium. These elements are also essential in our daily diet and are often supplemented. Importantly, multivalent ions such as Mg, Ca, and Fe have similar sizes and charges, which allows them to compete mutually during absorption, thereby affecting bioavailability and metabolism (Ford, Mokdad 2023). Recommended dietary allowance (RDA) is 400 mg for Mg (Ford, Mokdad 2023) and 1300 mg for Ca (Kennedy, Meyers 2005).

The data in Table 1 indicate that 100 g of animal-derived food can provide iron levels significantly above the daily requirement, which may have negative health consequences. As noted in the introduction, no more than 35% of total iron is absorbed, and only if it is in the heme form. For non-heme iron, this figure is no more than 20%. Therefore, we can assume that most animal products, except blood and beef spleen, provide adequate iron per 100 grams. The iron content in products like beef spleen and blood significantly exceeds the daily requirement, and consuming 100 g of these products can have adverse effects on our bodies. Nevertheless, we can consider these products a natural iron supplement. One level teaspoon of powdered beef spleen (~1.5 g) contains about 14 mg of iron, while powdered blood (~2.5 g) contains about 10 mg of Fe. On the other hand, plant-based foods do not meet this element's requirement; moreover, they lack the equally important zinc entirely. Animal products contain significantly less magnesium and calcium than plant products. In particular, pumpkin seeds are considered a rich source of magnesium. It should also be noted that the daily serving of spirulina and chlorella is approximately 3 g, so these products should not be considered a rich source of such elements as magnesium, calcium, and especially iron. While this may seem obvious, it is worth reading labels carefully when selecting specific products. The content of a given element in 100 g of the product may not be equivalent to the manufacturer's recommended daily allowance.

Figures 3 and 4 present selected Mössbauer spectra obtained for the animal and plant food products, respectively. The hyperfine parameters of the spectral components (quadrupole doublets), isomer shift (IS), and quadrupole splitting (QS) are summarized in Table 2. The isomer shift arises through an electric monopole interaction between the positive nuclear charge and the electric field of the surrounding electrons. This interaction causes a shift of the nuclear energy levels compared to an unperturbed nucleus, where the magnitude of the shift is a function of the difference in s-electron densities between the source and absorber nuclei (McCammon 2000). The value of isomer shift relates to the oxidation state of the nucleus, its spin state, and its bonding properties. The isomer shift is particularly sensitive to the spin state of the iron atom, and based on it, we can conclude about the oxidation state of the iron ion in the tested material. Quadrupole splitting arises through a quadrupole interaction between the nuclear quadrupolar moment and the electric field gradient at the nucleus due to the surrounding electrons, causing a splitting of the nuclear energy states (McCammon 2000).

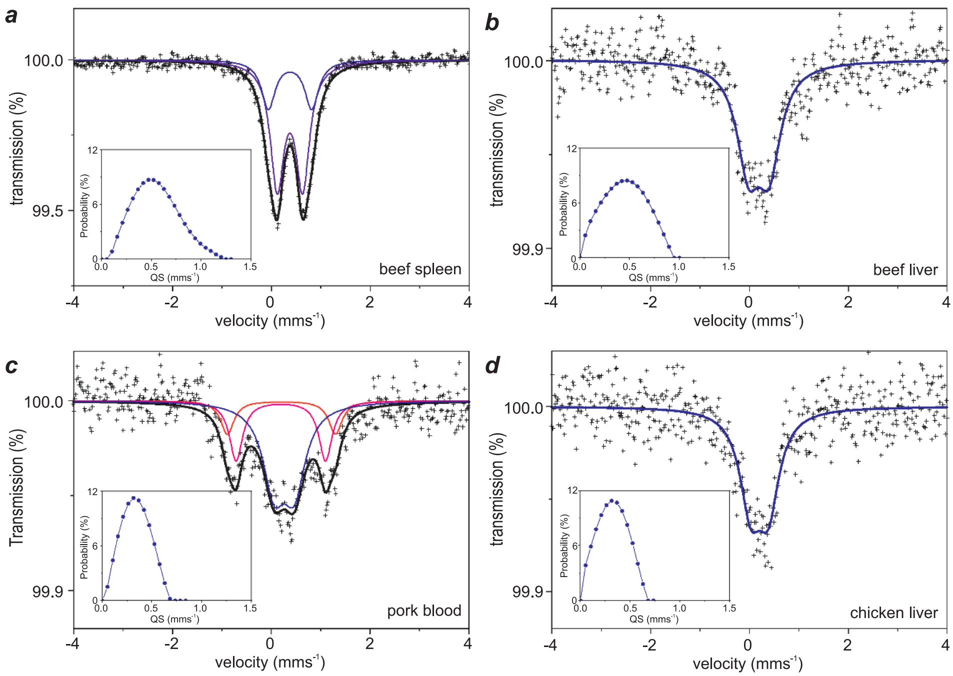

Fig. 3. The Mössbauer spectra obtained for selected animal products: beef spleen – *a*, beef liver – *b*, pork blood – *c*, and chicken liver – *d*. The experimental points, fitting curves, and spectral components are presented. Quadrupole splitting distributions for Fe^{3+} ions are presented as inserts

This parameter depends on the valence and spin state of the absorber atoms, as well as the coordination and degree of distortion of the crystallographic site. Since most of the spectra showed a symmetric quadrupole doublet with broad lines, they were also analyzed using the quadrupole splitting distribution (QSD). The QS distributions obtained were presented in the spectra as inserts. Both analyses yielded similar hyperfine parameters. The agreement between these parameters indicates that they can be assigned physical significance regardless of the fitting model used. The spectra of the samples for which asymmetric quadrupole splitting distributions (beef spleen and pork liver) were obtained were fitted using discrete two-component analysis. It is also important to note that all QS distributions were broad, resulting in broad absorption lines (Table 2). This resonance line broadening is referred to as inhomogeneous broadening (Stoneham 1969) and results from the presence of the Fe ion near different vacancy clusters (Barberan et al. 1979). The different field gradients produced by these clusters around the Fe ion cause quadrupole splitting of the resonance line with slightly different QS values.

The Mössbauer spectra of beef and pork blood samples were matched with three doublets. Two are associated with Fe^{2+} ions in deoxyhemoglobin

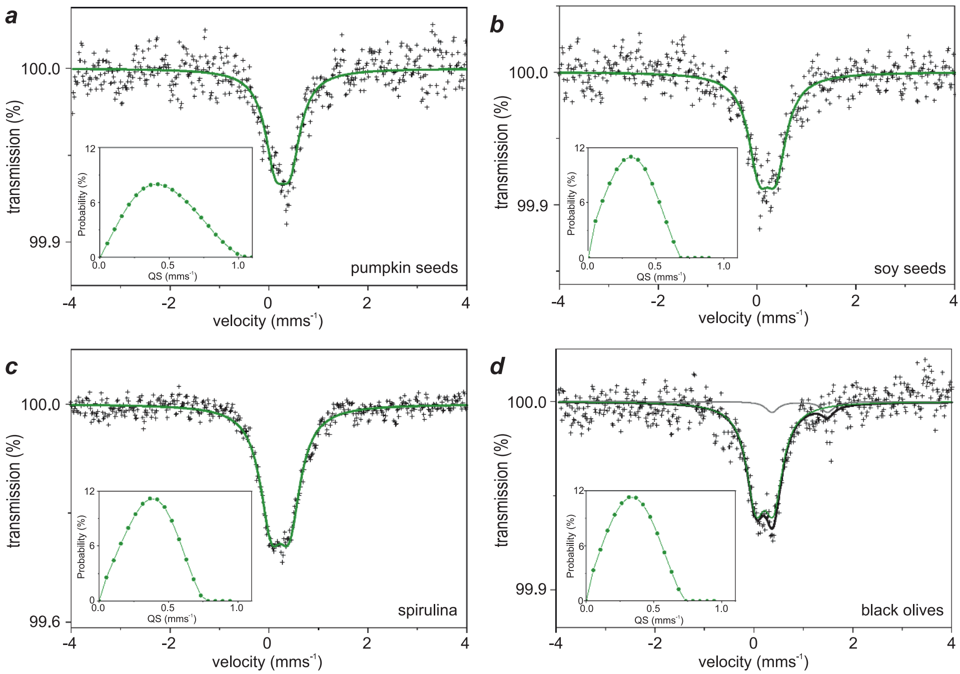

Fig. 4. The Mössbauer spectra obtained for selected plant products: pumpkin seeds – *a*, soy seeds – *b*, spirulina – *c*, and black olives – *d*. The experimental points, fitting curves, and spectral components are presented. Quadrupole splitting distributions for Fe^{3+} ions are presented as inserts

(HbO_2), and the third, associated with Fe^{3+} ions, has hyperfine parameters indicative of carboxyhemoglobin (HbCO) – Hoy et al. (1986), Ding et al. (1988), Bauminger et al. (1991). The Mössbauer spectra of beef and pork spleen and pork liver samples were fitted with two ferric doublets. These doublets have similar isomer shift values but differ in quadrupole splitting values. These two components are associated with Fe ions in ferritin (Mohie-Eldin et al. 1995, Longo et al. 2023), an iron storage protein found primarily in the liver, spleen, and bone marrow. The two different QS values of these doublets (Table 2) are associated with the fact that ferritin is made of two types of subunits: heavy chains (H) and light chains (L), which are arranged with specific symmetries and have distinct roles in iron oxidation and nucleation (Szymulewska-Konopko et al. 2025). The Mössbauer spectra of the remaining samples were fitted with a single ferric doublet. The hyperfine parameters of this doublet indicate the presence of Fe^{3+} ions in carboxyhemoglobin. On the other hand, we remember that iron is found in the human body in hemoglobin, which is present in red blood cells, and in myoglobin, which is found in muscles. Hemoglobin and myoglobin are proteins that store and transport oxygen. Although both proteins contain a heme group, they differ in location and function. Therefore, in the products in

Table 2

Mössbauer hyperfine parameters obtained for components of animal and plant food products

IS (mm s^{-1})	QS (mm s^{-1})	A (%)	G (mm s^{-1})	Fe oxidation state
Beef spleen				
0.38(1)	0.52(1)	72(1)	0.34(1)	Fe^{3+}
0.38(1)	0.88(3)	28(1)	0.34(1)	Fe^{3+}
Pork spleen				
0.38(1)	0.58(2)	69(1)	0.45(3)	Fe^{3+}
0.36(2)	1.22(4)	21(1)	0.50(5)	Fe^{3+}
Beef liver				
0.19(2)	0.42(3)	100(1)	0.55(5)	Fe^{3+}
Pork liver				
0.33(1)	0.36(3)	42(1)	0.40(3)	Fe^{3+}
0.38(1)	0.70(2)	58(1)	0.40(3)	Fe^{3+}
Chicken liver				
0.20(1)	0.36(3)	100(1)	0.53(4)	Fe^{3+}
Pork heart				
0.22(1)	0.43(3)	100(1)	0.60(4)	Fe^{3+}
Beef blood				
0.15(2)	0.38(3)	48(1)	0.55(5)	Fe^{3+}
0.22(3)	1.74(4)	10(1)	0.41(4)	Fe^{2+}
0.20(2)	2.02(4)	42(1)	0.41(4)	Fe^{2+}
Pork blood				
0.26(2)	0.41(3)	59(1)	0.55(3)	Fe^{3+}
0.19(1)	1.81(3)	26(1)	0.32(3)	Fe^{2+}
0.21(2)	2.19(3)	15(1)	0.32(3)	Fe^{2+}
Black olives				
0.22(2)	0.36(3)	93(1)	0.45(3)	Fe^{3+}
0.93(2)	1.13(3)	7(1)	0.30(2)	Fe^{3+}
Soy seeds				
0.21(1)	0.34(3)	100(1)	0.52(4)	Fe^{3+}
Pumpkin seeds				
0.24(3)	0.47(2)	76(1)	0.51(2)	Fe^{3+}
0.33(3)	-	24(1)	0.30(3)	Fe^{3+}
Chlorella				
0.26(1)	0.45(2)	100(1)	0.58(4)	Fe^{3+}
Spirulina				
0.23(1)	0.37(1)	100(1)	0.54(2)	Fe^{3+}

Explanations: IS – isomer shift, QS – quadrupole splitting, G – full line width at half maximum, A – area fraction of subspectra. The error in the least significant digit is given in brackets which we identified heme iron, it could have been present not in hemoglobin but in myoglobin, which has a greater affinity for oxygen; hence, only its oxidized form, Fe^{3+}, is observed. The hyperfine parameters of the Mössbauer spectra of animal products (Table 2) indicate that iron in these products occurs primarily in the heme form. Only beef and pork spleen, as well as pork liver, contain iron that can be considered nonheme.

The Mössbauer spectra of plant samples were also fitted with one ferric doublet, except for the spectrum obtained for black olives, where an additional component related to Fe^{2+} ions is visible. This component may be related to iron compounds added to the brine, which give black olives a more intense color. Unfortunately, we were unable to find hyperfine parameters reported for plant products in the literature. However, we can note that the ferric doublet observed in the spectra of plant products has hyperfine parameters that are very similar to those obtained for carboxyhemoglobin in animal products (Table 2). This component may be related to leghemoglobin, a heme-containing protein responsible for transporting oxygen in plants (Bolmanis et al. 2023, Świątek et al. 2023).

CONCLUSIONS

Iron deficiency is one of the most common diseases worldwide, occurring when the diet fails to meet the daily iron requirement. Our study analyzed animal and plant products commonly considered rich in iron and other essential minerals for proper bodily function. Using methods such as X-ray fluorescence analysis, we determined the content of critical macro- and micronutrients in the selected food products. The presented research also demonstrated the feasibility of using methods such as Mössbauer spectroscopy. The results of this technique allow us to characterize the chemical states of iron naturally occurring in food products. Figure 5 shows the values of hyperfine parameters for the components obtained for Mössbauer spectra of all investigated products. Iron in the animal products studied was primarily in the heme form. Only beef, pork spleen, and pork liver contained iron in the form of ferritin, an iron-storing protein. These three products contained nonheme iron. In a well-balanced diet, not only the amount of iron but also its oxidation state and the form in which it is administered are important. Heme iron has higher bioavailability than nonheme iron, but only when iron is in the Fe^{2+} form. Our results showed that Fe^{2+} ions were present only in the blood samples. Considering these results and the Fe content in all products studied (as determined by the XRF method), it can be said that one hundred grams of the investigated animal products contained sufficient iron to meet the daily requirement for this element. Consuming larger amounts of red meat is unnecessary. Due to the very high iron content in products such as beef spleen and beef and pork blood, these foodstuffs can

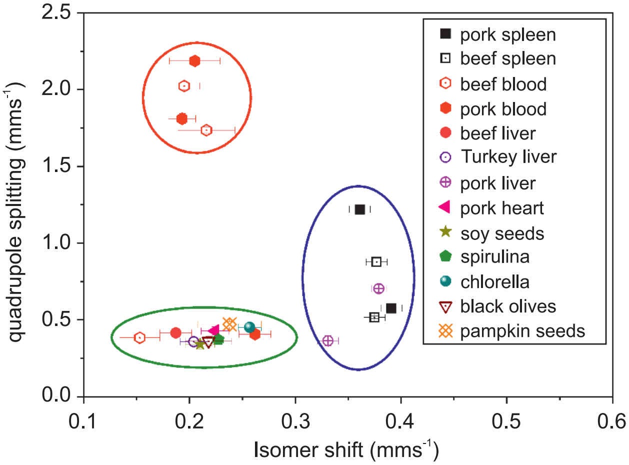

Fig. 5. Hyperfine parameters of the Mössbauer spectra of the investigated food products, where the red circle contains the characteristic parameters for Fe^{2+} in deoxyhemoglobin, the olive circle – Fe^{3+} in carboxyhemoglobin or leghemoglobin (plant products), and the blue circle – Fe^{3+} in ferritin

be considered natural iron supplements. Their cost will be several dozen times lower than the price of their synthetic counterparts. Moreover, animal products also contain other essential macro- and micronutrients, including zinc, potassium, magnesium, and calcium. The iron content in the plant products studied was significantly lower than in other plant products and cannot be considered a sufficient source of iron. Iron ions in these products are in their third oxidation state. A diet based solely on plant foods requires additional iron supplementation. On the other hand, plant products are richer in elements such as magnesium and calcium than animal products.

Author contributions

Mariola Kądziołka-Gaweł – conceptualization, supervision, formal analysis, investigation, writing – original draft preparation, writing – review & editing, Marcin Wojtyniak – investigation. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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