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wchodzących w skład przedstawianego osiągnięcia naukowego
oraz oświadczenia współautorów
wskazujące na ich merytoryczny wkład w powstanie każdej pracy**

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- [H1] **Puścion-Jakubik A.**, Socha K., Borawska M.H. *Comparative study of labelled bee honey from Poland and the result of the melissopalynological analysis*. Journal of Apicultural Research, 2020, 59(5), 928-938.
- [H2] **Puścion-Jakubik A.**, Karpińska E., Moskwa J., Socha K. *Content of phenolic acids as a marker of Polish honey varieties and relationship with selected honey-quality-influencing variables*. Antioxidants, 2022, 11(7), ID 1312.
- [H3] **Puścion-Jakubik A.**, Bielecka J., Grabia M., Markiewicz-Żukowska R., Soroczyńska J., Teper D., Socha K. *Comparative analysis of antioxidant properties of honey from Poland, Italy and Spain based on the declarations of producers and their results of melissopalinological analysis*. Nutrients, 2022, 14(13), ID 2694.
- [H4] **Puścion-Jakubik A.**, Olechno E., Socha K., Zujko M.E. *Eating habits during the COVID-19 pandemic and the level of antibodies IgG and FRAP – experiences of Polish school staff: a pilot study*. Foods, 2022, 11(3), ID 408.
- [H5] **Puścion-Jakubik A.**, Markiewicz-Żukowska R., Naliwajko S.K., Gromkowska-Kępka K.J., Moskwa J., Grabia M., Mielech A., Bielecka J., Karpińska E., Mielcarek K., Nowakowski P., Socha K. *Intake of antioxidant vitamins and minerals in relation to body composition, skin hydration and lubrication in young women*. Antioxidants, 2021, 10(7), ID 1110.

- [H6] **Puścion-Jakubik A.** †, Bielecka J. †, Grabia M., Mielech A., Markiewicz-Żukowska R., Mielcarek K., Moskwa J., Naliwajko S.K., Soroczyńska J., Gromkowska-Kępka K.J., Nowakowski P., Socha K. *Consumption of food supplements during the three COVID-19 waves in Poland – focus on zinc and vitamin D*. *Nutrients*, 2021, 13(10), ID 3361. †contributed equally
- [H7] **Puścion-Jakubik A.**, Kus K., Socha K. *Medical university students' perspective on marketing of dietary supplements*. *Acta Poloniae Pharmaceutica Drug Research*, 2021, 78(2), 205-218.
- [H8] **Puścion-Jakubik A.** †, Bartosiewicz N. †, Socha K. *Is the magnesium content in food supplements consistent with the manufacturers' declaration?* *Nutrients*, 2021, 13(10), ID 3416. †contributed equally
- [H9] **Puścion-Jakubik A.** †, Staniaszek G. †, Brzozowska P., Socha K. *Quality of calcium food supplements: evaluation compared to manufacturers' declarations*. *Molecules*, 2022, 27(23), ID 8154. †contributed equally
- [H10] **Puścion-Jakubik A.**, Mielech A., Abramiuk D., Iwaniuk M., Grabia M., Bielecka J., Markiewicz-Żukowska R., Socha K. *Mercury content in dietary supplements from Poland containing ingredients of plant origin: a safety assessment*. *Frontiers in Pharmacology*, 2021, 12, ID 738549.

Publikacja H1



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Comparative study of labelled bee honey from Poland and the result of the melissopalynological analysis

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ORIGINAL RESEARCH ARTICLE

Comparative study of labelled bee honey from Poland and the result of the melissopalynological analysis

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The definition of a variety of natural bee honey is based on a melissopalynological analysis of the pollen grains that are the most prevalent and naming the variety of honey according to the botanical name of the plant. The purpose of this work was to compare the declarations of beekeepers regarding a variety of bee honey from Poland with the results of laboratory analysis. The study was conducted on 100 samples of bee honey. It was shown that 48% of the samples were incorrectly classified in the declared variety. Only heather honey samples showed 100% correct classification. In contrast, 7% of rape honey, 13% of buckwheat, 58% of multifloral, 68% of linden, 100% of dandelion and 100% of willow honey were wrongly named. The consumer, when looking for a specific variety of bee honey, expects health benefits, which is why individual parameters defining the quality of bee honeys, especially varieties, should be the subject of detailed and continuous monitoring.



Keywords: botanic authenticity; pollen grains; melissopalynology; honey

Introduction

Bee honey is mostly a mixture of sugars (blossom honey – average content: 69.5%, honeydew honey: 57.9%) and water (respectively: 17.2% and 16.3%), but also contains many compounds with nutritional and prophylactic properties, including minerals (0.2% and 0.9%), enzymes, amino acids and protein (0.3% and 0.6%), acids (0.5% and 1.1%),

vitamins, essential oils, dyes and phenolic compounds (Bogdanov, 2016; Wilde, 2013).

The classification of honey for a variety, according to the Regulation in force in Poland, is as follows: the percentage share of pollen grains originating from individual plant species is determined. From the name of the plant, from which the percentage of pollen in honey is

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in significant advantage, it is called the variety of honey (Dz. U. 2009 No. 17 item 94). Another classification, previously in force, required a minimum amount of pollen grains from a given plant to call honey varietal: in Poland in rape honey should be not less than 45% of *Brassica napus* L. pollen grains, in buckwheat honey – not less than 45% of pollen grains from *Fagopyrum esculentum* Moench, in heather honey – not less than 45% of pollen grains from *Calluna vulgaris* (L.), in *Robinia pseudoacacia* L., in linden honey – not less than 20% of pollen grains from *Tilia* sp. L. (Polski Komitet Normalizacji, Miary i Jakości, 1988).

Beekeepers determine the botanical origin of their honey mainly on the basis of organoleptic characteristics – such as colour, taste, smell and consistency. For example, heather (*Calluna vulgaris* L.) honey is characterized by a gelatinous, medium-grained consistency; buckwheat (*Fagopyrum esculentum* Moench) honey has a sharp taste, whereas black locust (*Robinia pseudoacacia* L.) honey, before crystallization, is colourless, and after crystallization has the colour of straw (Polski Komitet Normalizacji, Miary i Jakości, 1988). Secondly on the basis of bee flight patterns and the flowering time of honey plants. Commercially available bee honey, in accordance with national and European legislation, should be of a high quality and meet a number of qualitative and quantitative criteria, not only regarding, for example, water, proline, 5-hydroxymethylfurfural, but also in terms of the proportion of pollen (Council Directive, 2002; Dz. U. 2003 No. 181 item 1773; 2015 item 850; 2010 No. 165 item 1120; International Honey Commission, 2009; Dyrektywa 2014/63/UE; World Health Organization & Food & Agriculture Organization of the United Nations, 1987).

The classification of over one hundred honey plants used by bees for the production of monofloral honey in Europe, carried out by Oddo et al. (2004), emphasizes that in the case of Polish honeys, these plants are: heather (*Calluna vulgaris* (L.) Hull) – abundant amount, buckwheat (*Fagopyrum esculentum* Moench) – abundant amount, lime (*Tilia* spp.) – abundant amount and white clover (*Trifolium repens* L.) – average amount.

The need for frequent checks and analysis of the honey variety, as well as the search for quick methods that will allow the variety to be determined, is caused by the lack of conformity of the beekeeper's declaration with the result of melissopalynological analysis. This is confirmed by, among others the Inspectorate of Agricultural and Food Quality (IjHARS) research, which showed large discrepancies as to the marking of the variety, which misleads the consumer. During the inspection of natural bee honey in 2012, irregularities in the variety were demonstrated in 45.5% of honey (Inspekcja Jakości Handlowej Artykułów Rolno-Spożywczych, 2013), and in 2013, irregularities were found in 37.5% of examined honey (Inspekcja Jakości Handlowej Artykułów Rolno-Spożywczych, 2014).

Melissopalynology is a branch of palynology, which aims to perform pollen analysis of bee products. This method allows the determination of the geographical and botanical origin of honeys even if sensory and physicochemical analyses are also needed for a correct diagnosis of botanical origin (Dybova-Jachowicz & Sadowska, 2003). The aim of this technique is, among others, to determine the amount of predominant pollen, whose proportion is larger than that of other pollen present in the microscopic preparation (Dz. U. 2009 No. 17 item 94). Pollen pellets are the basis for determining the variety of bee honey corresponding to the botanical name of the plant. It also serves to assess preferences in terms of visited plants.

It should be emphasized that the determination of the botanical origin of natural bee honey is based on the relative incidence of pollen grains in honey sediment. As a general rule, monoflower honey is classified when the relative incidence of taxon pollen is above 45%. Individual plants, however, differ in the level of abundance of pollen grains in nectar, hence there are exceptions to this rule. This is due to the fact that in the case of too few represented pollen grains, the amount of nectar that actually participates in the formation of honey is greater than that resulting from the number of pollen grains (honey from *Tilia* sp. L., *Robinia pseudoacacia* L.), and in the case of over-represented pollen grains – is smaller (*Castanea* Mill. honey is over-represented, so its sediment must contain at least 90% grains from this species to be considered varietal). An important phenomenon is also secondary enrichment (being pollen from the hive), tertiary (accumulation during honey extraction) and quaternary (air pollution) (Von Der Ohe et al., 2004).

In addition, it is worth emphasizing that pollen grains present in bee honey provide information on vegetative changes in plants occurring in a given geographical area, which can be used for their analysis (Dybova-Jachowicz & Sadowska, 2003).

The melissopalynological method, the basics of which were developed by Pfister, began in 1895 (Maurizio and Hodges, 1951). Melissopalynological analysis in Poland has been developed since the 1960s by Demianowicz and Demianowicz's (1957), based on Louveaux, Maurizio, and Vorwohl (1978) work, who developed a methodology for qualitative and quantitative honey analysis. They introduced the nomenclature for pollen grains depending on frequency: "very frequent" – "predominant pollen" (for grains in excess of 45% in total), "frequent" – "secondary pollen" (for grains from 16 to 45%), "rare" – "important minor pollen" (for grains from 3 to 15%) and "sporadic" – "minor pollen" (for grains less than 3%).

In addition, this technique is useful for determining the falsification of honey, e.g., Polish honey, which was obtained from other countries. For example, for Chinese honey, the presence of pollen grains from *Polygala* L. is characteristic (Teper, 2004). The presence of pollen grains also confirms whether honey is natural

Table 1. The correctness of the classification of honey types by Polish beekeepers.

Variety of honey	Classification according to Dz. U. 2009 No. 17 item 94		Classification according to Beckg & Camps, 2009	
	Properly classified (%)	Improperly classified (%)	Properly classified (%)	Improperly classified (%)
Buckwheat	87	13	87	13
Dandelion	0	100	0	100
Heather	100	0	100	0
Lime	32	68	32	68
Multifloral	42	58	42	58
Rape	93	7	27	73
Willow	0	100	–	–

or adulterated, and whether it originates from another geographical area.

In the literature, there are classifications of natural bee honey as monofloral (when the pollen grains from one plant exceed 45%), bifloral (when the share of pollen grains from two plants was 22.45%) and multifloral (when pollen grains from more than 3 botanical species account for less than 16%) (Chauhan, Farooqui, & Trivedi, 2017; Wingenroth, 2001). The above classification was used in the presented publication, modifications were also applied, taking into account the above described exceptions.

The specific electrical conductivity of bee honey is the conductivity determined for 1 ml of a 20% honey solution, calculated on the dry mass (m/m). The test consists of measuring the electrical resistance using a conductivity cell (Dz. U. 2009 No. 17 item 94).

The aim of the study was to compare the declarations of beekeepers regarding the variety of natural bee honey from Poland with the results of laboratory analysis, based on the determination of predominant pollen.

Materials and methods

One-hundred (100) samples of natural bee honey, classified by beekeepers as: multifloral ($n = 33$), lime (*Tilia* spp. L.) ($n = 22$), buckwheat (*Fagopyrum* spp. Moench) ($n = 15$), rape (Brassicaceae) ($n = 15$), dandelion (*Taraxacum* spp. F. H. Wigg) ($n = 8$), heather (*Calluna* spp. (L.) Hull) ($n = 4$), and willow (*Salix* spp. L.) ($n = 3$) were obtained in Poland: at a fair with organic food and bee products, in bee food stores, in supermarkets and directly from beekeepers.

The melissopalynological analysis was carried out according to the *Recommendations of the International Commission for Bee Botany of IUSB* – the method described by Louveaux et al. (1978) and Von Der Ohe et al. (2004) *Harmonized methods of melissopalynology* – this is in line with the law in force in Poland – *Regulation of the Ministry of Agriculture and Rural Development, Journal of Laws 2009 No. 17 item 94 as amended* (Dz. U. 2009 No. 17 item 94 as amended, Dz. U. 2015 item 1173). 10 g of honey was weighed and mixed well, 20 ml of distilled water heated to 40 °C was added, thoroughly mixed till dissolved. The resulting solution was centrifuged at 3000 rpm for 10 minutes, after decanting the supernatant liquid, 20 ml of distilled

water was added to completely dissolve the remaining sugar crystal and then centrifuged again for another 5 minutes. The soak was collected: if the precipitate was 0.1 ml, a 0.5 cm thick layer of solution was left over, and when the precipitate was 0.3 ml, a 1 cm layer was left. From this suspension, 0.1 ml was taken and placed evenly on a base slide covering a 1 cm² area (Dz. U. 2009 No. 17 item 94 as amended). The microscope preparation was dried and then fixed. In the formulation, a uniform distribution of pollen grains on the slide was evaluated. 300 consecutive pollen grains were then qualified for botanical type or species. Various publications recommend for the classification 300, 600 or other numbers of pollen grains. To correctly classify honey for a variety, the rule of counting 300 consecutive pollen grains in a microscopic preparation applies in Poland – this principle was applied to be able to compare the results with the results obtained by other authors researching honeys in Poland. From 300 grains, pollen grains of nectarless and wind-pollinated plants (for example corn – *Zea* L., hazel – *Corylus* L., St. John's wort – *Hypericum* L., poppy – *Papaver* L.) were subtracted and the amount of predominant pollen (%p) was calculated according to the formula:

$\%p = (n_p \times 100)/z$ (Dz. U. 2009 No. 17 item 94 as amended, Dz. U. 2015 item 1173), where n_p – the number of pollen grains presenting a significant advantage over pollen from other plants, z – the number of pollen grains of nectariferous plants (Dz. U. 2009 No. 17 item 94 as amended, Dz. U. 2015 item 1173).

The above method is recommended by law in force in Poland regarding the determination of bee honey variety (Dz. U. 2009 No. 17 item 94 as amended, Dz. U. 2015 item 1173). The analyses were performed using a Delta Optical microscope (magnification: 400x).

Two repetitions were carried out for each preparation when the difference between the two analyses was lower than and/or equal to 5%. When the difference was above 5%, more repetitions were performed (Dz. U. 2009 No. 17 item 94 as amended).

In addition, confirmation of the type of honey (nectar, nectar-honeydew, honeydew) was made using conductometric analysis. The electrical conductivity was determined by the conductometric method, using a conductometer Mettler Toledo. Honey (20 g, calculated on the dry

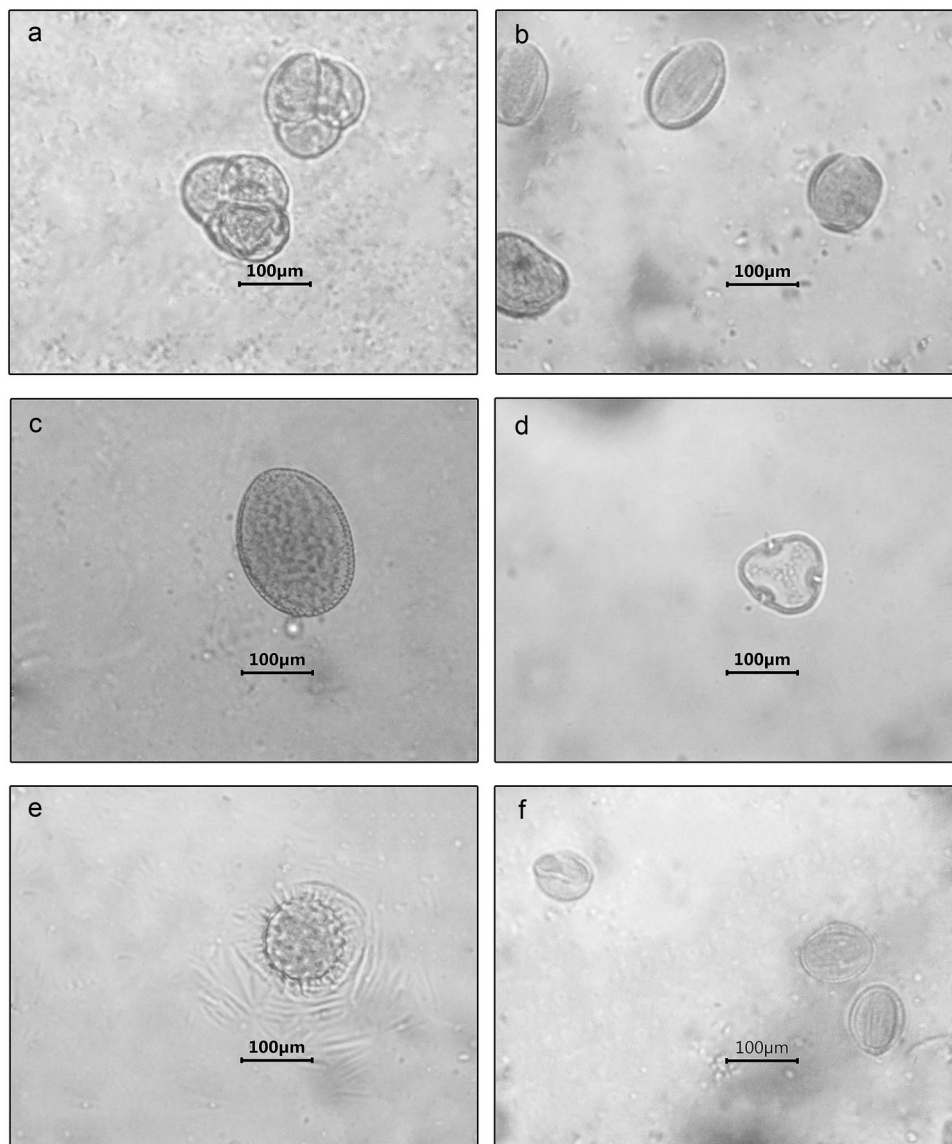


Figure 1. a–e. Microscopic photographs (magnification: 400x, scale: 100 µm) of pollen grains. **Ia.** *Calluna vulgaris* (L.) Hull. **Ib.** *Brassica napus* L. subsp. *napus*. **Ic.** *Fagopyrum esculentum* Moench. **Id.** *Tilia* L. **Ie.** *Taraxacum officinale* F. H. Wigg. **If.** *Salix alba* L.

matter) was weighed, dissolved in distilled water and up to 100 ml. Solution (40 ml) was withdrawn, brought to a temperature of 40 °C and the electrical conductivity was measured. For each sample, the measurement was performed three times (Dz. U. 2009 No. 17 item 94 as amended).

Results

Studies have shown that a total of 48% of the samples of natural bee honey were not in accordance with the declarations of beekeepers (Table I).

Since the tested honeys were on sale in Poland, the applicable provisions were adopted as the classification criterion for the variety: the share of pollen grains from a given plant in a significant amount, the exception was linden honey: the share of pollen grains from *Tilia* spp. L. – above 20% (Dz. U. 2009 No. 17 item 94 as amended). In addition, the results obtained were also

referred to international guidelines that require: for honeys from *Brassica napus* L.: more than 80% of pollen grains (pollen normally represented but slightly over-represented, average 80%, tolerance 70%), for honeys with *Tilia* sp. L. – above 20% (pollen under-represented, average >20% tolerance 10%), for honeys with *Calluna vulgaris* (L.) Hull. – 2–90% due to extraction, for honeys with *Taraxacum* sp. F. H. Wigg. – above 15% (pollen under-represented in honey) and for honeys with *Fagopyrum* sp. Moench – above 30% (Beckh & Camps, 2009).

The greatest accuracy of classification, comparing individual varieties of honey, was demonstrated for heather (100%), rape (93%) and buckwheat (87%) honeys (Table I). In the case of heather honey 100% of the samples (Table I) were in accordance with the declaration, with a predominance of pollen grains from heather (*Calluna vulgaris* (L.) Hull) (Figure 1a). In the case of honeys labelled as rape, 93% of the samples (Table I)

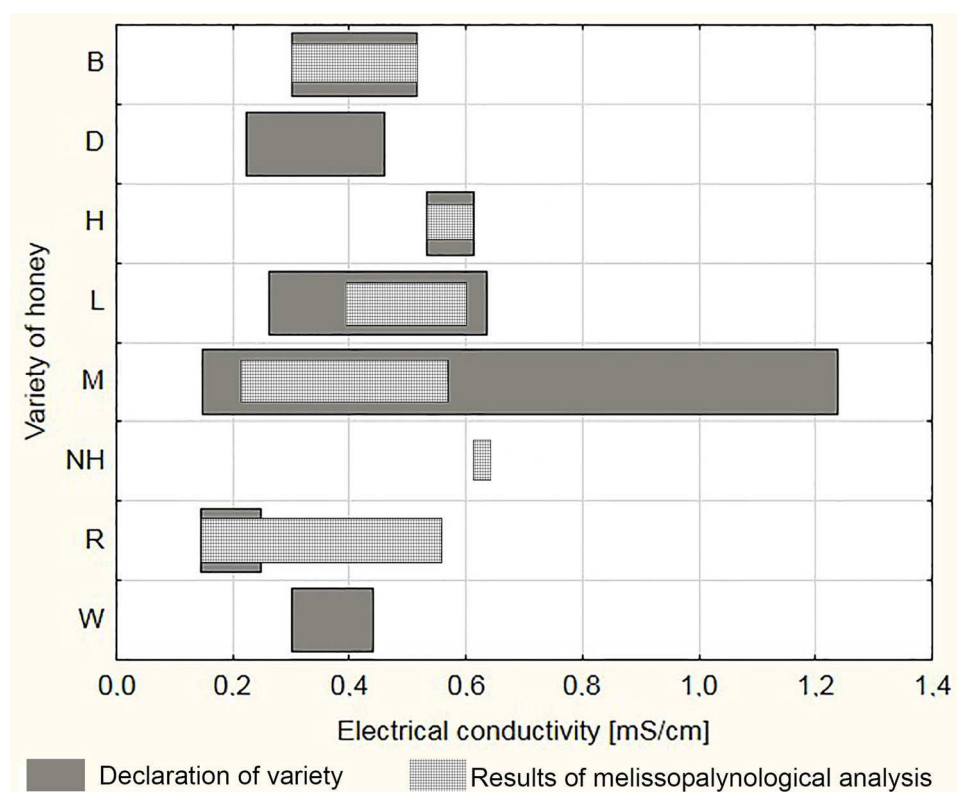


Figure 2. Electric conductivity of natural bee honey (B – buckwheat honey, D – dandelion honey, H – heather honey, L – lime honey, M – multifloral honey, NH – nectar-honeydew honey, R – rape honey, W – willow honey).

Table 2. Scope of share of pollen, average and standard deviation, in properly defined varieties of the studied natural bee honeys.

Variety of honey	Predominant pollen [%]				
	Average \pm SD	Min – Max	Median	Q ₁	Q ₃
Buckwheat	54.4 \pm 11	46 - 81	48	47	58
Heather	65.3 \pm 16	50 - 82	65	52	79
Lime	23.1 \pm 4	20 - 30	22	20	27
Rape	62.2 \pm 16	46 - 96	58	48	71

Q₁, Q₃ – quartile 1 and 3

contained predominant pollen grains from *Brassica napus* L. var. *napus* (Figure 1b); one honey should be classified as multiflorous honey. Based on the guidelines described above (Beckh & Camps, 2009), only 27% of honeys had sufficient content of guiding pollen. Among buckwheat honey, 87% of honey (Table 1) showed compliance with the declaration of beekeepers, it contained mostly pollen grains from buckwheat (*Fagopyrum esculentum* Moench) (Figure 1c), but 2 samples of honey should be classified as multifloral. Only 32% of lime honey (Table 1) was characterized by the correctness of classification (predominance of pollen grains derived from linden *Tilia* L.) (Figure 1d); 7 samples of honey should be classified as multifloral honey, 6 as rape (despite the fact that linden honey is very characteristic in sensory analysis and very different from rapeseed honey), and 2 as nectar-honeydew (honeydew lime is a common additive in honeys nectar). All of the analysed dandelion and willow honey (Table 1) were characterized as containing too low of an amount of pollen grains from dandelion (*Taraxacum*

officinale F. H. Wigg) (Figure 1e) and willow (*Salix alba* L.) (Figure 1f) to be identified as dandelion and willow honey. It was also shown that 58% of multifloral honeys (Table 1) were characterized by incorrect classification botanical origin: 15 samples should be classified as rape, 2 as buckwheat, 1 as nectar-honeydew and 1 as honeydew (due to characteristic microscopic image and the value of electrical conductivity) (Figures 1 and 2).

The average share of pollen grains from *Tilia* L. in lime honey, in samples with confirmed botanical origin, was 23.1 \pm 4%, from *Brassica napus* L. subsp. *napus* in rape honey – 62.2 \pm 16%, from *Fagopyrum esculentum* Moench in buckwheat honey – 54.4 \pm 11%, while from *Calluna vulgaris* (L.) Hull in heather honeys – 65.3 \pm 16%. Classification into varieties was carried out on the basis of Louveaux et al. (1978), with the exception of lime honeys, which were classified in accordance with the laws in force in Poland, which require at least 20% of pollen grains coming from lime to be present (Polski Komitet Normalizacji, Miar i Jakości, 1988) (Table 2).

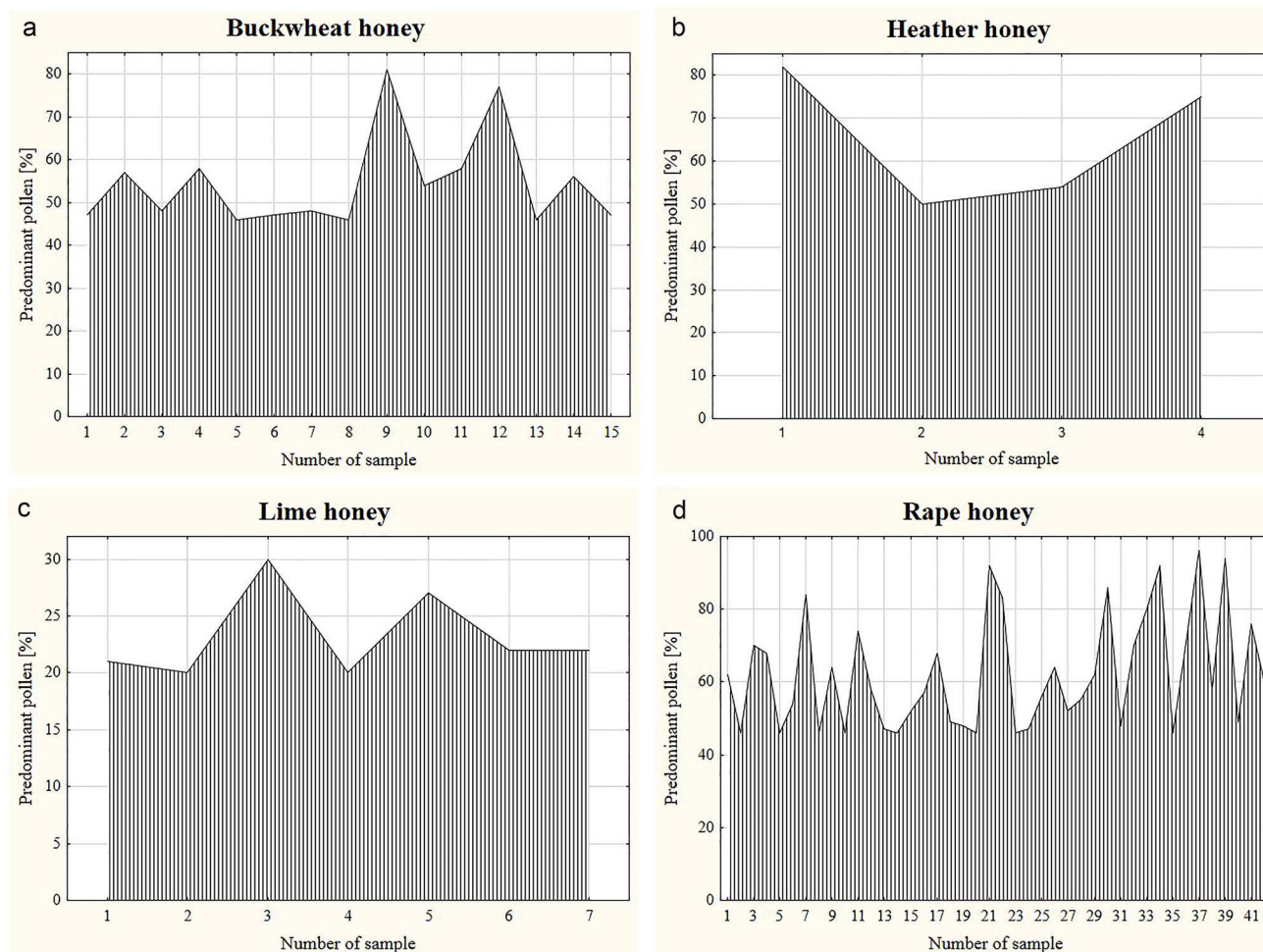


Figure 3. The predominant pollen in the examined natural bee honey samples: a – buckwheat honey, b – heather honey, c – lime honey, d – rape honey.

The amount of predominant pollen in the tested samples varied: for buckwheat honey from 46 to 81%, for heather honey from 50% to 82%, for linden honey from 20% to 30% and for rape honeys from 46 to 96% (Table 2, Figure 3a–d).

For multifloral honey, detailed pollen analysis was also performed (Table 3). It has been shown that in this honey from Poland is dominated by pollen grains of white clover – *Trifolium repens* L. (19%), rape – *Brassica napus* L. (17%) and buckwheat – *Fagopyrum esculentum* Moench (17%). Other pollen grains include, among others, pollen from elderberry – *Sambucus nigra* L. (9%), forget-me-not – *Myosotis* L. (8%), St. John's wort – *Hypericum perforatum* L. (7%) and *Cardamine pratensis* L. (5%). Additionally, in the available literature (Wilde, 2013), the periods of flowering of individual plants were checked – such an assessment was carried out to check whether the lack of conformity of the manufacturer's declaration with the result of the palynological analysis may result from accidental honey beating by the bee-keeper or from deliberate activities. In the case of analysed multifloral honey, the flowering time of different plant species may indicate that the nectar was collected by bees between May and June.

Measurements of electrical conductivity were carried out for all types of honey being investigated. The work presents graphs (Figure 2) – grey fields show the electrical conductivity depending on the variety declared by the manufacturer, while white rectangle in black check – depending on the melissopalynological analysis carried out in the current study ($n = 100$).

According to the regulations in force, COUNCIL DIRECTIVE 2001/110/EC of 20 December 2001 relating to honey, the electrical conductivity for nectar honeys should not exceed 0.8 mS/cm, for honeydew honey: more than 0.8 mS/cm – exceptions are e.g., honey from linden (*Tilia* spp.) and common heather (*Calluna vulgaris*) – however, they were analysed in this paper to evaluate this parameter for the above varieties. It has been shown that one honey does not meet this requirement. The Polish Standard, previously in force in Poland, additionally delimited the electrical conductivity of nectar honey to no less than 0.2 mS/cm, nectar-honeydew – no less than 0.4 mS/cm, of honeydew honeys – no less than 0.8 mS/cm, and with conifer honeydew – no less than 0.95 mS/cm (Polski Komitet Normalizacji, Miary i Jakości, 1988).

The average value of electrical conductivity in honeys (classified after melissopalynological analysis)

Table 3. Pollen analysis of selected multifloral honeys with the flowering time of individual plants.

Plant name	Frequency of occurrence (%) ^a	Flowering time ^b
White clover – <i>Trifolium repens</i> L.	19	Blooms at the end of V, blooms all summer
Rapeseed cabbage – <i>Brassica napus</i> L.	17	Winter forms - bloom in the 1st half of V, spring forms: from the beginning of VII
Buckwheat – <i>Fagopyrum esculentum</i> Moench	17	Blooms: beginning of VII, flowering time: up to 7 weeks
Elderberry – <i>Sambucus nigra</i> L.	9	Flowering time: end of V - to VI
Forget-me-not – <i>Myosotis</i> L.	8	Blooms: I half of V, blooms to the end of VIII
St. John's wort – <i>Hypericum perforatum</i> L.	7	Flowering time: VI - VIII
Cuckooflower – <i>Cardamine pratensis</i> L.	5	Flowering time: V or VI
Apple tree – <i>Malus</i> Mill. type	4	Blooms: first decade of V, flowering time: 2-3 weeks
Bluebonnet – <i>Centaurea cyanus</i> L. type	3	Blooms: I half of VI, flowering time: to harvest
White dead-nettle – <i>Lamium album</i> L.	2	Flowering time: end of IV - to the end of summer
Berberis – <i>Berberis</i> L.	1	Blooms in V
Celandine – <i>Chelidonium majus</i> L.	1	Flowering time: V - IX
Wild strawberry – <i>Fragaria</i> L.	1	Blooms in V and VI
Campion – <i>Lychnis flos-cuculi</i> L.	1	Blooms in V and VI
Bird cherry – <i>Padus</i> Mill. type	1	Blooms in the 3rd decade of V
Tare – <i>Vicia</i> L. type	1	Blooms in VI and VII

^aOwn results.^bBased on Wilde (2013).

was 0.396 ± 0.06 mS/cm for buckwheat honey, 0.567 ± 0.04 mS/cm for heather honey, 0.518 ± 0.08 for lime honey, 0.268 ± 0.11 for rape honey and 0.395 ± 0.10 for multifloral honey.

Additionally, a correlation analysis was performed between the amount of predominant pollen and electric conductivity. It was shown that there is a significant inverse relationship between these parameters ($r = -0.40$, $p < 0.01$). The results showed no significant correlations between the measured parameters within individual varieties. The strongest correlation occurs in linden honey ($r = -0.58$), in which the average share of the predominant pollen is the lowest ($23.1 \pm 4\%$), while the weakest in heather honey ($r = -0.02$), which is characterized by the largest share of the predominant pollen ($65.3 \pm 16\%$). Interestingly, the share of pollen in rape honey was $62.2 \pm 16\%$, but the correlation coefficient was lower ($r = -0.15$).

Discussion

Consumer interest in bee honey, as an element that supports the treatment of many diseases, is constantly increasing, as well as the demand for different varieties

of honey each with its own specific properties. The required minimum average proportion of pollen grains in the honey settlement depends on the amount of pollen produced. For example, *Castanea* spp. produces a very large amount of easily pollinating pollen to nectar and the forget-me-not has a characteristic flower structure: the bee carries all the pollen from anther to nectar (Wilde, 2013). According to *The Polish Standard* (Polski Komitet Normalizacji, Miary i Jakości, 1988), the minimum percentage of the pollen of the reference taxon required for linden honey is at least 20%, for *Robinia* 30%, for rape and buckwheat - at least 45% (Dz. U. 2003 No. 181 item 1773 as amended). Honey from other geographical areas has different requirements: for example, for lavender honeys from Portugal, the share of pollen, which allows the classification of honey as a monofloral, is only 15% (Silva, Sousa & Taveira, 2017), whereas honey obtained from *Brassica napus* L. in Croatia should contain at least 60% of pollen grains from this plant, and in Germany - at least 80% (Thrasyvoulou et al., 2018).

The publications available in the scientific literature on melissopalynological analysis, cited below, are usually based mainly on the determination of characteristic taxa

and percentage of pollen grains, and not on the comparison of the variety declaration with the variety determined in laboratory conditions, therefore this work focuses on this topic (Lecewicz, 1979; Poszwiński & Warakomska, 1969; Stawiarz, 2012; Stawiarz, Wróblewska, Kozłowski, & Masierowska, 2017).

Poszwiński and Warakomska (1969) conducted research on rape and heather honey from Poland, obtained from beehives, with the exception of the possibility of contamination with foreign pollen. In rape honey, they showed *Brassica* as predominant pollen at the level of 62–93% (while in the current study from 46 to 96%), in heather honey *Calluna* pollen range from 42 to 79% (similar results were obtained in this study: from 50 to 82%). The secondary pollen in rape honey came from *Castanea*, *Salix* and fruit trees, in heather honey: pollen grains from white and red clover, buckwheat, weed from the Brassicaceae family. Some pollen grains occurred as a small percentage, but in the majority of samples. They characterize plants that are the feed base for bees and allow the determination of the period of nectar harvesting.

Lecewicz (1979), examining honey samples obtained in Poland in 1959, 1961 and 1962, at the beginning of the development of this method, showed that among buckwheat honey only one has too little pollen of *Fagopyrum*, which is much lower than in studies carried out in the current work (13%). In other buckwheat honeys, the percentage share of grains was from 40.5% to 85.9%. In addition, for these honey samples, it was shown that the number of other plant species that occurred in honey was from 3 to 19.

Stawiarz (2012) analysed honeys from the Świętokrzyskie region of Poland. He revealed that 50% of varietal honeys showed compliance with the requirements of the Polish Standard regarding the share of pollen. The presented work shows a similar percentage of compliance with the beekeeper's declaration as to the variety with the result of melissopalynological analysis (52%). Samples derived from fruit trees, from *Trifolium* L., *Phacelia* Juss., *Galeopsis* L., *Sinapis alba* L. and *Salix* L., were wrongly classified. The author identified pollen grains from 29 nectarless plant species and 80 nectar-secreting plants in 143 honey samples. Further research, published in 2017 (Stawiarz et al., 2017), included various bee products. In all 9 honey samples there were pollen grains from 52 taxa belonging to 28 botanical families, including 36 from nectariferous plants and 16 from non-nectariferous ones: the majority were Asteraceae, Fabaceae and Rosaceae. This research allowed the characterization of flora, useful for bees, from the Świętokrzyskie Mountains region.

Researchers at the Laboratory of Bee Quality Research in Puławy, Institute of Horticulture and the Institute of Beekeeping, have characterized the most popular varieties of Polish honey. They showed that the percentage of reference taxon for both linden honey and

heather honey was higher than in the present study and was respectively $28.1 \pm 8\%$ (Wąs, Rybak–Chmielewska, Szczesna, Kachaniuk, & Teper, 2011a) and $69.0 \pm 20\%$ (Wąs, Rybak–Chmielewska, Szczesna, Kachaniuk, & Teper, 2011b). Rape pollen percentage was lower than in this paper: $52 \pm 7\%$ (Szczesna, Rybak–Chmielewska, Wąs, Kachaniuk, & Teper, 2011).

The analysis showed that the average content of the lime pollen percentages was $23.1 \pm 4\%$, whereas Tucak, Periškić, Škrivanko, and Konjarević (2007) showed a higher percentage of pollen grains obtained from Croatia – the average content of pollen grains was 36%.

In this paper, we have shown that 48% of natural samples of bee honeys are characterized incorrectly. Earlier studies, carried out in our laboratory on honey from 1998, showed that 43% of the samples were incorrectly classified (Piekut, Witkowska, Borawska, & Hejft, 2000). Analysis of the Polish honey, carried out on a smaller number of samples ($n = 45$) showed that over one third of the declared varieties (36%) were incompatible with the results of microscopic analysis (Puścion-Jakubik & Borawska, 2016).

The Inspectorate of Agricultural and Food Quality, mainly dealing with, the quality assessment of bee honeys, annually publishes the results of its inspections. In 2007, 147 parties were inspected. It was shown that 35% of honeys were incorrectly labelled (Inspekcja Jakości Handlowej Artykułów Rolno-Spożywczych, 2007). The 2008 report showed, however, that 18% of the honeys were incorrectly named (Inspekcja Jakości Handlowej Artykułów Rolno-Spożywczych, 2008). This result is much lower than that of our research, although the research method used in this work was the same.

Additionally, the Inspectorate of Agricultural and Food Quality issued 2 administrative decisions confirming adulteration of bee honeys: the decision of May 19, 2015, specifies that a batch weighing 486.18 kg was incorrectly labeled as buckwheat honey (it did not have enough buckwheat pollen) (Decyzja Głównego Inspektora Jakości Handlowej Artykułów Rolno-Spożywczych, 2015). The decisions issued on 30 October, 2013, regarding a 23.25 kg batch (Decyzja Głównego Inspektora Jakości Handlowej Artykułów Rolno-Spożywczych, 2013) show that *Robinia* honeys were wrongly classified, as *Robinia* pollen was only at a level of 16.2 and 17.4%. The honeys contained great amounts of rape pollen.

Insect-pollinating plants, which are extremely important for apiculture, and are mentioned in the literature, include St. John's Wort (*Hypericum* L.), rose (*Rosa* L.), celestron herb (*Chelidonium majus* L.) and beetle (*Filipendula* Mill.) (Wilde, 2013). However, acacia, lime, buckwheat, rape, heather, multifloral, nectar-honeydew and honeydew honeys are most frequently available in Poland and are often bought by consumers. Analysis of the selected natural bee honey (Table 3) showed that pollen grains from plants blooming from early May to the end of summer were present. The selected plants

may exhibit a number of prophylactic properties. For example, pollen grains derived from St. John's wort (*Hypericum perforatum* L.) accounted for 7%. Mirmalek et al. (2016) confirmed the cytotoxic activity of hypericin, the main component of this plant, on the MCF-7 breast cancer cell line. The presence of hypericin and biflavonoids has been indirectly confirmed in pollen grains by Hölscher, et al. (2009).

Conductivity is mainly determined by the presence of minerals, ash, organic acids and proteins (Habib, Al Meqbali, Kamal, Souka, & Ibrahim, 2014; Lazerević, Andrić, Trifković, Tešić, & Milojković-Opsenica, 2012). A previously published article (Puścion-Jakubik & Borawska, 2014) contained data on the correlation between colour intensity and electrical conductivity. A negative correlation was found between these parameters for linden honey, buckwheat honey and heather honeys (in presented work they have a higher conductivity – on average from 0.396 to 0.567 mS/cm), positive for rape honey honeys (in this study the average conductivity is 0.268 ± 0.11 mS/cm). Dark honeys are richer in mineral so they have higher conductivity, instead of pale honeys have a lower conductivity since they contain few minerals. However, a negative correlation can be explained, according to Persano Oddo, Piazza, Sabatini, and Accorti (1995), as the “stronger” properties of some nectars. It was shown that even a small number of nectar from heather, chestnut, *Fagopyrum* or honeydew can change the physicochemical properties of honey. The high conductivity of dark honey with low protein content can also be explained due to the large size of their molecules, which causes the release of ion motions (Andrełowicz & Kotlarek, 1968).

Majewska et al. (2017) examining buckwheat honey from Poland ($n = 17$), showed that all samples meet the requirements specified by law, and the range of values was from 0.110 to 0.499 mS/cm, while the average result in this work was 0.396 mS/cm. Statistical analyses carried out by researchers aimed at searching for botanical similarity depending on physicochemical parameters. It has been demonstrated by means of cluster analysis that parameters such as electrical conductivity, water, ash, protein and sucrose belong to one group.

It is worth noting that honeys from Lithuania Kaškonienė, Venskutonis and Čeksteryte (2010), showed a negative correlation between the specific electrical conductivity and the content of rapeseed pollen grains ($r = -0.90$), and a positive one in willow honey ($r = 0.90$). It was a much higher correlation than demonstrated in the presented work.

As demonstrated in the current study, almost half of commercially available honeys from Poland were incorrectly classified when taking into account their varieties. Among the reasons for such a high percentage of non-compliance of the beekeeper's declaration as to the variety in comparison with the results of the laboratory analysis could be secondary contaminated honey in

honeycombs (Stawiarz, 2012), accidental pollen sprinkled during the harvesting of the final product, lack of valid laboratory analyses and deliberate falsification.

The melissopalynological method is currently recommended by legislation, but it may be erroneous, therefore the results of the analysis should be interpreted with caution. Secondary enrichment of honey in pollen grains may already have taken place in the hive, tertiary – during extraction from the patch, while third/another – may be pollutants from the air (Von Der Ohe et al., 2004).

Conclusions

Consumers, looking for natural honey, expect certain nutritional and preventive benefits, depending on the botanical origin of the variety. The conducted research has shown that as many as 48% of honeys available for sale are categorized as an incorrectly specified variety. Pollen analysis is extremely important to prove the botanical origin of honey. Therefore, the quality of bee honeys, and especially their variety, determined by the melissopalynological method and the conductometric method, should be constantly monitored before being placed on the market.

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Publikacja H2



Article

Content of Phenolic Acids as a Marker of Polish Honey Varieties and Relationship with Selected Honey-Quality-Influencing Variables

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Abstract: Phenolic acids are an important component of honey. Literature data indicate their pro-health properties and diversified content in different varieties. Therefore, the aim of our study was to evaluate the content of phenolic acids in bee honey. The material for the research was 49 samples of honey obtained from beekeepers from Poland. Selected phenolic acids were determined by HPLC with PDA detection. Additionally, total phenolic content (TPC), color intensity, color on the Pfund scale, water content, electrical conductivity, and FRAP were assessed. A higher trans-ferulic acid content is accompanied by a stronger free radical scavenging ability. It was shown that buckwheat honeys are characterized by a high TPC value (196.59 mg GAE/100 g), color intensity (2109.2 mAU), color on the Pfund scale (159.8 mm Pfund), and high activity in the FRAP assay (0.403 equivalent of $\mu\text{mol Fe}^{2+}/\text{mL}$). The median obtained in the DPPH test for this honey variety was 41.1%. Moreover, the highest median of 4-hydroxybenzoic acid (3.129 mg/100 g) in buckwheat honey was shown. Buckwheat honeys have promising antioxidant properties and should be included in diets low in antioxidants.

Keywords: honeybee; buckwheat honey; Poland; markers; phenolic acids



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1. Introduction

Bee honey is a product of very diverse composition. It includes, among others, sugar compounds, water, proteins, organic acids, vitamins, minerals, phenolic compounds, enzymes, and many other ingredients [1]. Honey available for sale should be properly labeled, including, inter alia, the name of the variety. Beekeepers define a variety based on the color, consistency, smell, taste or on the basis of observation of the plants from which the bees collect nectar or honeydew.

Earlier publications indicate that a large percentage of honey is incorrectly labeled [2]. The classic method for determining the type of honey is the melissopalinalogical method, which consists of counting pollen grains under a microscope and classifying them into botanical species. This is a time-consuming method that requires detailed observation of the grains. Sometimes, it is emphasized that its results are ambiguous and difficult to interpret. Therefore, other methods of identifying honey varieties are being sought. For example, an electronic potentiometric tongue has been developed to help identify the honey variety [3]. The nuclear magnetic resonance (NMR) method was used to distinguish between nectar and honeydew honey [4]. Another method that can be used to identify honey varieties is the method of fluorescence spectroscopy. It was used to distinguish, among others, acacia, linden, and sunflower honey [5].

Other methods of honey classification are based on searching for characteristic markers or identifying fingerprints. For example, high-performance liquid chromatography with diode array detection and tandem mass spectrometry (HPLC-DAD-MS/MS) was used to

distinguish between chaste honey and rape honey. The following markers were considered: ferulic acid, kaempferol, and morin. Additionally, chromatographic fingerprints at 270 nm and 360 nm were identified. The above methods were used in conjunction with chemometric techniques [6]. An attempt to identify the honey variety on the basis of its antioxidant properties was also undertaken by Džugan et al. (2018). Buckwheat honey had the strongest antioxidant properties, and rape honey had the weakest [7].

In addition, the health-promoting properties of bee honey may be conditioned by the presence of compounds with antioxidant properties, including phenolic acids. The literature describes many beneficial properties of bee honey, including its use in the treatment of burns and ulcers [8], rosacea [9], acute cough [10], and bedsores [11]. HPLC is one of the most popular methods used to determine the content of individual compounds with antioxidant properties [12].

Phenolic substances, which are phenol derivatives, are synthesized by plants. They are divided into simple phenols and polyphenols. Polyphenols contain more than one hydroxyl in their molecule structure. Polyphenols can exist in free form or in combination with other substances, such as glycosides (made of aglycone and sugar residue). Phenolic acids include compounds derived from cinnamic and benzoic acids, including caffeic acid, ferulic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, protocatechuic acid, syringic acid, and vanillic acid. They can interact with biologically active molecules and protect them against damage [13]. In addition to phenolic acids, the antioxidant properties of honey are due to, among others, flavonoids, vitamins (such as vitamins C and E), and minerals (including zinc and manganese) [1]. For example, the literature data indicate that the most common flavonoids in acacia honeys are: apigenin, chrysin, galangin, genistein, kaempferol, luteolin, myricetin, pinobanksin, pinocembrin, and quercetin. Those characteristic of manuka honey are: chrysin, galangin, isorhamnetin, kaempferol, luteolin, pinobanksin, pinocembrin, and quercetin [14].

Therefore, the aim of the research was to assess whether selected phenolic acids can be a marker of individual varieties of honey from Poland, as well as to correlate the content of these acids with selected parameters determining the quality of the honey, such as color scale, color intensity, total phenolic content, water content, electrical conductivity, and % free radical scavenging in DPPH assay.

2. Materials and Methods

2.1. Materials

The research material consisted of 49 samples of natural bee honeys: buckwheat ($n = 15$), linden ($n = 9$), multi-flower light ($n = 3$), dandelion ($n = 4$), nectar–honeydew ($n = 4$), rape ($n = 8$), honeydew ($n = 3$), and heather ($n = 3$). Honey was purchased in Poland; each sample was made by a different beekeeper. Until analysis, the honeys were stored at 4 °C.

All solvents were HPLC grade, and all chemicals were analytical and reagent grade. Formic acid (min. 98%) was obtained from Merck (Darmstadt, Germany). Methanol was purchased from J.T. Baker (Avantor, Gliwice, Poland).

Ultrapure water was obtained from Simplicity™185 Water Purification System (Merck Millipore, Darmstadt, Germany).

HPLC standards of polyphenols such as: 3,4-dihydroxybenzoic acid (3,4-DHBA), 4-hydroxybenzoic acid (4-HBA), caffeic acid (CA), *p*-coumaric acid (*p*-CA), syringic acid (SA), trans-ferulic acid (*t*-FA), vanillic acid (VA), and reagents for determining the total content of phenolic compounds (Folin–Ciocalteu reagent, Na₂CO₃) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Individual stock solutions of each analyte and a mixture of them were prepared in methanol.

2.2. Methods

2.2.1. Identification of the Varieties of Honey

The classification of variety was made on the basis of melissopalinalogical analysis, in accordance with the Regulation of the Minister of Agriculture and Rural Development [15]. From each honey, 10 g was weighed in a centrifuge tube, supplemented with 50 °C water to 20 mL, mixed, and centrifuged for 10 min at 3000 rpm. The precipitate was decanted, water was added again, and centrifuged. When the sediment was about 0.1 cm, a layer of water of about 0.5 ml was left above it, and when it was about 0.3 cm - a layer of about 1 ml of water was left, a homogeneous suspension was obtained and applied to a microscope slide. At least two microscopic preparations were made of each honey, in which pollen grains were classified to botanical varieties. On the basis of the grains present in the greatest predominance in given bee honey, each was given a variety name.

2.2.2. Determination of Water Content

Honey in an amount of 5 g was weighed into a test tube, closed with a stopper, and placed in a water bath from 45 °C until brought to a liquid state. Then, a few drops of honey were placed in the refractometer, and the refractive index was read. In the case of temperatures above 20 °C, the factor was increased by 0.00023/1 °C, and in the case of temperatures below 20 °C, it was reduced in a similar manner. Then, the water content was read from the table in the Regulation. For each honey, at least 2 determinations were made. The results are expressed in % [15].

2.2.3. Determination of Electrical Conductivity

Based on the water content of each honey, the amount to be weighed was calculated according to the following formula:

$$M = \frac{20 \text{ g} \times 100}{MS},$$

where:

M —the mass of honey to be weighed (g),

MS —dry matter content, calculated as the difference between 100% and the water content, expressed as %.

The honey was weighed out and made up to 100 mL with distilled water. The conductivity cell was rinsed with the sample, and a honey solution (40 mL) was placed in a water bath at a temperature of 20 °C; when the temperature of the solution was 20 °C, the electrical conductivity was measured. The electrical conductivity of honey was calculated according to the formula:

$$S = K \times G, \text{ where :}$$

SH —specific conductivity of honey ($\text{mS} \times \text{cm}^{-1}$),

K —constant of the conductivity cell (cm^{-1}),

G —conductivity (mS).

2.2.4. Determination of Color Intensity

In order to determine the color intensity, 5 ± 0.001 g of honey was weighed, and water at 45 °C temperature was added at a volume of 10 g and mixed thoroughly. The solution was then sonicated and filtered through a 0.45 μm filter. The absorbance of the solutions was measured at 450 and 720 nm. The final result was the difference in absorbance at the two wavelengths, expressed in mAU. For each sample, three determinations were performed, and the final result was the mean result [16].

2.2.5. Determination of Color on the Pfund Scale

To determine the color of natural bee honey using the Pfund scale, 5 ± 0.001 g of sample was weighed, the samples were each dissolved into 10 mL of distilled water, and

they were mixed well. The samples were then placed in a water bath at 50 °C to dissolve the sugar crystals. After obtaining a clear solution, absorbance was measured at 635 nm against distilled water. The Pfund color scale was calculated using the formula:

$$\text{mm Pfund} = -38.70 + 371.39 \times \text{Absorbance}.$$

The final result is the average of three measurements [17].

2.2.6. Determination of Total Phenolic Content (TPC)

The total content of phenolic compounds was determined by reaction with the Folin–Ciocalteu reagent [18]. A calibration curve was prepared using a gallic acid working solution with a concentration of 2 g/L. A 1 ± 0.001 g sample was taken from each honey. Honey was dissolved in distilled water to a volume of 10 mL and then centrifuged at 2000 rpm for 5 min. Next, 0.25 mL of supernatant was collected; then, 1.25 mL of 0.2 N Folin–Ciocalteu was added, and the sample was stirred for 5 min. Next, 1 mL of Na_2CO_3 solution was added, mixed, and incubated in the dark at room temperature for 2 h. The contents of each tube were then mixed, and the absorbance at 760 nm against water was measured using a Hitachi U-2001 spectrophotometer. The results are presented as the mean of 3 determinations, in mg gallic acid/100 g honey.

2.2.7. Determination of Radical Scavenging Activity by DPPH Assay

The ability of bee honeys to scavenge radicals was performed on the basis of the method described by Sánchez-Moreno et al. [19]. Bee honeys were dissolved in distilled water to obtain a concentration of 1 g/mL. In total, 200 μL was taken, and 1800 μL of a DPPH solution with a concentration of 0.04 mg/mL was added. The absorbance at 517 nm was measured with a spectrophotometer U-2001 (Hitachi, Tokyo, Japan). The samples were then incubated at room temperature, protected from light, for 30 min. After the incubation period, the absorbance was measured again. The % of free radical scavenging was calculated:

$$\text{DPPH [\%]} = \left[\frac{A_0 - A_{30}}{A_{30}} \right] \times 100\%,$$

where A_0 is the absorbance at time 0, and A_{30} is the absorbance over 30 min.

2.2.8. Determination of FRAP

To perform the FRAP test, the FRAP reagent was prepared (2.5 mL of a 10 mM TPTZ solution in 40 mM HCl, 2.5 mL of 20 mM FeCl_3 , and 25 mL of 0.3 M acetate buffer pH 3.6) [20].

To 20 μL of honey solution, 180 μL of FRAP reagent was added, and the mixture was incubated at 37 °C for 10 min. The absorbance of the mixture was then measured at 593 nm with a plate reader (UVM 340, Biogenet, Józefów, Poland). The results are presented as the equivalent of $\mu\text{mol Fe}^{2+}/\text{mL}$ of the sample [20].

2.2.9. Preparation of Samples for HPLC Analysis–Isolation of Phenolic Compounds

Honey samples (5 g) were dissolved in 50 mL of water (adjusted to pH 2 with HCl) until completely fluid. This solution (50 mL) was then filtered through a Sep-Pak C18 cartridge (tube type SPE, Supelclean LC-18 SPE Tubes 3 mL/500 mg, Supelco Analytical, Bellefonte, PA, USA), which was previously activated with methanol (10 mL) followed by water (10 mL). The phenolic compounds were retained on the column, whilst all sugars and other polar compounds were eluted with water, and then polyphenols were eluted with 2.5 mL of a methanol–water mixture (70%, *v/v*) in order to validate the efficiency of extraction SPE and similar activities dealing with standards.

Phenolic fractions in methanol evaporated under reduced pressure (22 °C). The residue was redissolved in a mixture of distilled water and HPLC-grade methanol, in proportions such as phase (22.5 MeOH parts: 76.5 H_2O parts: 1 CH_3COOH parts). The prepared sample

was analyzed by HPLC with photodiode array (PDA) detection. The applied extraction method enabled recovery values for analyzed compounds of higher than 85%.

2.2.10. HPLC Analysis

HPLC analyses of honey extracts were performed using an Flexar HPLC system (Perkin Elmer, Waltham, MA, USA) with a photodiode array detector (PDA) and using Chromera LC-PDA software (Perkin Elmer, Waltham, MA, USA). Separations were carried out with reversed-phase column Synergi 4 μ m C-18 (Merck, Darmstadt, Germany; 250 \times 4.60 mm, particle size 4 micron, 80A), SecurityGuard Cartridges Fusion-RP 4 \times 3.0 mm ID. A mobile phase of 22.5 MeOH:76.5 H₂O:1 CH₃COOH was used; a constant solvent flow rate (1 mL/min) was applied. The total analysis time was 50 min. An isocratic separation method was used using the mobile phase (22.5 MeOH:76.5 H₂O:1 CH₃COOH). The temperature of the column oven was set at 25 °C. The phenolic acids were detected at 254, 265, and 326 nm, since the most honey phenolic compounds show their UV absorption maxima around these three wavelengths. The comparison of UV spectra and retention times with standard compounds enabled the identification of phenolic acids presented in the analyzed honey extracts. These compounds were quantified against their external standards. The injection volume was 20 μ L.

Each sample was analyzed three times, and the method was proved by repeatability test by determining peak area and retention reproducibility for different classes of compounds.

Table 1 presents data on the optimization of the method, including LOQ (limit of quantitation) and LOD (limit of detection).

Table 1. Characteristics of the developed method.

Compounds	RT	LOD (mg/100 g)	LOQ (mg/100 g)
3,4-DHBA	9.183	0.099	0.300
4-HBA	16.570	0.092	0.278
VA	20.284	0.089	0.271
CA	21.756	0.106	0.322
SA	23.886	0.147	0.445
<i>p</i> -CA	40.572	0.138	0.418
<i>t</i> -FA	50.040	0.084	0.255

3,4-DHBA—3,4-dihydroxybenzoic acid, 4-HBA—4-hydroxybenzoic acid, CA—caffeic acid, LOD—limit of detection, LOQ—limit of quantitation, *p*-CA—*p*-coumaric acid, RT—retention time, SA—syringic acid, *t*-PA—*trans*-ferulic acid, VA—vanillic acid.

The concentrations of 4-HBA, VA, and *t*-FA were read at 254 nm and 3,4-DHBA at 265 nm. However, the 326 nm wavelength was the best to read for CA, *p*-CA, and SA. During the optimization of the chromatographic conditions, the necessary quality parameters of the method were taken into account, including retention factors, relative retention factors, and resolution. The resolution of the compounds was 1.5 and above, with the exception of 3,4-DHBA, where the average resolution was 1.0–1.3.

2.2.11. Statistical Analysis

Statistical analyses were performed using Statistica v.13.3 software. Values of $p < 0.05$ were considered significantly different. The correlation between all the measured parameters was evaluated using Spearman's correlation coefficient.

In order to compare the values for several independent groups the Kuskal–Wallis ANOVA tests were performed.

Chemometric analyzes were also performed, including cluster analysis (CA) and principal component analysis (PCA). In the CA, agglomeration was chosen as the method of grouping. The agglomeration method is single bond, and the distance measure is Euclidean distance.

3. Results

3.1. Varieties of Bee Honey

The first analytical step was to assess whether the marking of honey by beekeepers was correct in order to correctly identify the compounds present in the tested honey at a later stage. We have shown that three of the honeys labeled as ‘buckwheat’ were of a different type. None of the dandelion honeys were of this variety. Among linden honeys, an incorrect declaration of variety was found in over 56% of the honey samples. Among nectar–honeydew honeys, one out of four samples should be marked differently (Table 2).

Table 2. The percentage of honey with the correct and incorrect definitions of the variety.

Variety–Declarations of Beekeepers	The Number of Attempts Correctly Classified	The Number of Attempts Is Classified Incorrectly
buckwheat ($n = 15$)	12	3
dandelion ($n = 4$)	0	4
heather ($n = 3$)	3	0
honeydew ($n = 3$)	3	0
linden ($n = 9$)	4	5
multi-flower light ($n = 3$)	3	0
nectar–honeydew ($n = 4$)	3	1
rape ($n = 8$)	8	0

Figure 1 shows pictures of pollen grains characteristic of buckwheat honey (Figure 1a), for heather honey (Figure 1b), for linden honey (Figure 1c), and for rapeseed honey (Figure 1d).

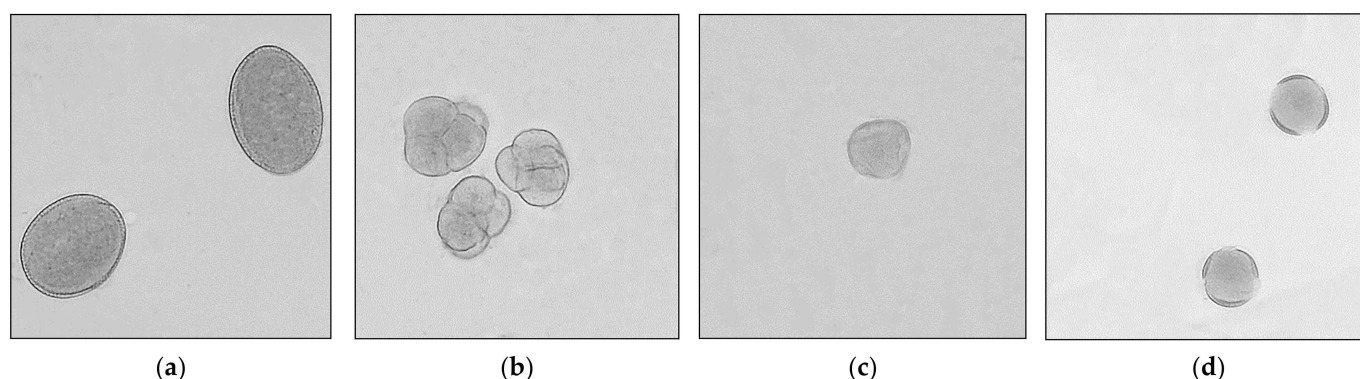


Figure 1. Pollen grains from honey plants: (a) *Fagopyrum esculentum* Moench, (b) *Calluna vulgaris* (L.) Hull, (c) *Tilia* L., (d) *Brassica napus* L. var. *napus*.

3.2. Selected Quality Parameters

Selected quality parameters examined as part of the quality assessment and the search for markers of honey from Poland are determination of the color of honey on the Pfund scale, determination of the color intensity, total phenolic compounds (TPC), water content, and electrical conductivity (Table 3).

We showed that buckwheat honeys were characterized by the highest color value on the Pfund scale (median: 159.8 mm Pfund)—this value was significantly higher compared to the color of linden (44.9 mm Pfund), multifloral light (37.4 mm Pfund), and rape honey (84.8 mm Pfund). A similar tendency was observed for the determination of color intensity: buckwheat honey (2109.2 mAU) had the highest median. Honey of this variety was also characterized by the highest TPC value (196.59 mg GAE/100 g), as well as the highest activity in the FRAP test (0.403 equivalent of $\mu\text{mol Fe}^{2+}/\text{mL}$). Interestingly, honeys of this variety have the ability to scavenge free radicals by about 41.1%. Honeydew honeys, on the other hand, showed the highest specific electrical conductivity ($1.181 \text{ mS} \times \text{cm}^{-1}$), significantly higher than that of rape honey ($0.242 \text{ mS} \times \text{cm}^{-1}$).

Table 3. The value of the parameters for individual varieties of honey.

Variety (Sign)	Colour Scale (mm Pfund)	Colour Intensity (mAU)	TPC (mg GAE/100 g)	Water Content (%)	Electrical Conductivity (mS \times cm ⁻¹)	DPPH (% Free Radical Scavenging)	FRAP (Equivalent of μ mol Fe ²⁺ /mL)
Buckwheat (B)	166.4 \pm 29.4	1816.0 \pm 688.0	182.60 \pm 61.08	18.9 \pm 0.5	0.400 \pm 0.043	42.0 \pm 4.5	0.402 \pm 0.010
	125.8–218.5	711.0–2634.7	44.95–241.87	18.1–19.9	0.326–0.507	34.9–52.7	0.379–0.417
	159.8	2109.2	196.59	18.9	0.391	41.1	0.403
	147.9–189.0	1229.0–2291.8	142.29–236.63	18.4–19.3	0.380–0.416	39.8–44.0	0.398–0.409
Heather (He)	125.2 \pm 14.8	575.8 \pm 179.5	91.78 \pm 4.25	19.2 \pm 0.7	0.552 \pm 0.027	46.4 \pm 3.7	0.141 \pm 0.002
	111.1–140.7	468.0–783.0	87.72–96.20	18.6–19.9	0.534–0.583	42.3–49.5	0.139–0.143
	124.0	476.3	91.42	19.0	0.538	47.5	0.140
	111.1–140.7	468.0–783.0	87.72–96.20	18.6–19.9	0.5334–0.583	42.3–49	0.139–0.143
Honeydew (Ho)	109.9 \pm 95.9	587.1 \pm 327.0	86.0 \pm 55.3	16.3 \pm 0.6	1.728 \pm 1.072	58.6 \pm 4.0	0.323 \pm 0.017
	49.8–220.5	215.3–830.0	42.8–148.3	15.7–16.8	1.041–2.963	55.9–63.2	0.312–0.343
	59.5	716.0	67.07	16.4	1.181	56.7 * B	0.315
	49.8–220.5	215.3–830.0	42.78–148.30	15.7–16.8	1.041–1.922	55.9–63.2	0.312–0.343
Linden (L)	43.5 \pm 19.6	84.0 \pm 44.0	29.23 \pm 10.60	16.7 \pm 0.7	0.502 \pm 0.104	58.6 \pm 1.4	0.083 \pm 0.012
	20.0–64.2	49.0–148.3	18.24–43.69	15.7–17.1	0.396–0.597	56.6–59.7	0.071–0.099
	44.9 ** B	69.3 *** B	27.50 ** B	16.9	0.508	59.0 ** B	0.081
	27.8–59.2	57.5–110.5	22.44–36.03	16.2–17.1	0.413–0.592	57.5–59.7	0.075–0.091
Multifloral dark (Md)	124.4 \pm 25.6	1424.7 \pm 803.1	187.6 \pm 194.3	19.2 \pm 0.8	0.416 \pm 0.026	56.7 \pm 2.8	0.218 \pm 0.015
	91.9–154.1	280.0–2160.0	55.60–467.83	18.1–20.0	0.380–0.437	53.2–59.3	0.198–0.232
	125.8	1629.3	113.50	19.3	0.423	57.2 * B	0.221
	107.3–141.5	953.7–1895.7	56.37–318.85	18.7–19.7	0.397–0.435	54.4–59.0	0.206–0.230
Multifloral light (MI)	33.3 \pm 24.3	155.6 \pm 90.9	32.04 \pm 3.80	18.6 \pm 0.7	0.431 \pm 0.109	45.3 \pm 6.5	0.052 \pm 0.032
	1.0–64.7	64.0–272.0	28.86–38.26	18.0–19.3	0.308–0.584	37.4–52.8	0.014–0.090
	37.4 *** B	128.0 * B	30.85 ** B	18.1	0.452	46.8	0.062 ** B
	18.9–44.2	85.0–229.0	29.44–32.79	18.1–19.3	0.344–0.466	39.8–49.6	0.023–0.070

Av. \pm SD
Min-Max
Med
Q1-Q3

Table 3. Cont.

Variety (Sign)	Colour Scale (mm Pfund)	Colour Intensity (mAU)	TPC (mg GAE/100 g)	Water Content (%)	Electrical Conductivity (mS \times cm ⁻¹)	DPPH (% Free Radical Scavenging)	FRAP (Equivalent of μ mol Fe ²⁺ /mL)
Nectar–honeydew (Nh)	115.2 \pm 55.2	322.6 \pm 231.7	57.08 \pm 11.54	17.8 \pm 1.7	0.641 \pm 0.031	57.4 \pm 4.2	0.205 \pm 0.010
	75.4–192.6	93.7–623.0	47.20–70.74	16.5–20.3	0.609–0.670	52.4–61.8	0.197–0.219
	96.5	286.8	55.19	17.2	0.642	57.8 * B	0.203
	75.5–155.0	145.8–499.3	47.51–66.65	16.8–18.8	0.614–0.667	54.1–60.8	0.198–0.213
Rape (R)	81.47 \pm 31.88	127.7 \pm 48.69	33.18 \pm 6.28	18.7 \pm 0.8	0.284 \pm 0.092	47.9 \pm 5.7	0.030 \pm 0.012
	17.5–125.6	62.0–231.0	20.40–43.94	17.7–20.6	0.169–0.449	37.8–58.7	0.012–0.058
	84.8 ** B	126.0 *** B	35.10 ** B	18.6	0.242 ** Ho, ** Nh	48.0	0.030 *** B
	66.4–98.8	86.0–150.3	30.17–36.74	18.1–19.1	0.215–0.352	45.6–52.3	0.022–0.035

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3.3. Profile of Phenolic Acids and the Variety of Honey

HPLC analysis showed the presence of seven phenolic compounds in honey from Poland: CA, *p*-CA, 3,4-DHEA, *t*-FA, SA, VA, and 4-HBA.

Figure 2 shows the chromatograms for standard substances at three wavelengths: 254 nm, 265 nm, and 326 nm.

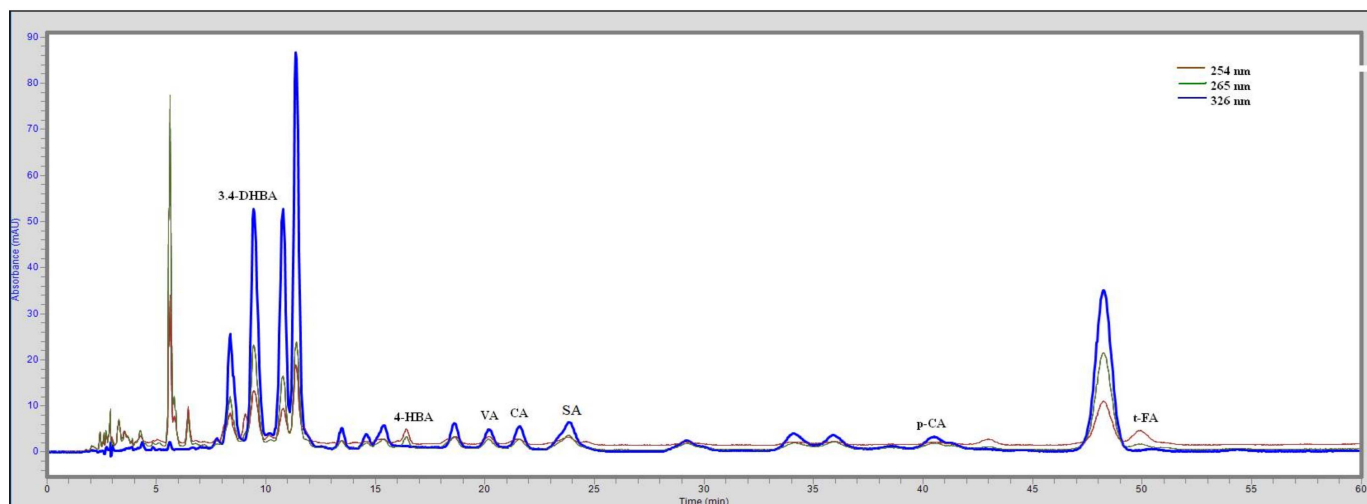


Figure 2. Chromatogram of the analyzed phenolic acid standards.

The calculated levels of individual identified phenolic compounds in analyzed honey are shown in Table 4. The ANOVA analysis of variance showed differences in the content of individual phenolic acids between the groups. Each of the varieties of honey is characterized by a high or low content of a specific phenolic compound.

It has been shown that the content of individual phenolic compounds for varieties of honey is characteristic. 3,4-DHBA was the highest median in linden (1.993 mg/100 g) and buckwheat (1.421 mg/100 g) honey. The next compound, 4-HBA, was characteristic for buckwheat (3.129 mg/100 g) and multifloral dark (1.934 mg/100 g) honey. The other determined phenolic acids such as CA, VA, SA, and *t*-FA were of highest value in linden honey (1.746, 0.304, 1.107, 1.954 mg/100 g, respectively). Moreover, *p*-CA was of a similar level to buckwheat (0.804 mg/100 g) and multifloral dark (0.789 mg/100 g) honey (Table 4).

In buckwheat honey, the highest median of 4-HBA was found—this value was significantly higher than that of the content in linden, multifloral light, nectar–honeydew, and rape. This indicates that the above compound can be considered a marker of the authenticity of buckwheat honey.

Another analyzed compound was 3,4-DHBA. Our study showed that linden honey had a significantly higher content of this phenolic acid than rape honey and CA compared to buckwheat honey.

Among the determined compounds, no characteristic concentrations were found for heather, honeydew, multifloral, nectar–honeydew, and rape honeys.

Table 4. The value of the phenolic acids for individual varieties of honey (mg/100 g).

Variety (Sign)	3,4-DHBA	4-HBA	CA	<i>p</i> -CA	VA	SA	<i>t</i> -FA
Buckwheat (B)	1.403 ± 0.419	3.203 ± 0.736	0.194 ± 0.073	0.784 ± 0.129	0.151 ± 0.043	0.186 ± 0.127	0.095 ± 0.050
	0.784–2.233	1.699–4.432	<LOD-0.325	0.558–1.004	<LOD-0.193	<LOD-0.329	<LOD-0.175
	1.421	3.129	0.207	0.804	0.165	<LOD	<LOD
	1.101–1.558	2.869–3.515	0.177–0.219	0.678–0.870	<LOD-0.180	<LOD-0.198	<LOD-0.152
Heather (He)	0.539 ± 0.056	0.895 ± 0.172	0.215 ± 0.025	0.386 ± 0.059	0.162 ± 0.143	0.860 ± 0.159	0.106 ± 0.092
	0.505–0.604	0.736–1.078	0.189–0.239	0.340–0.452	<LOD-0.273	0.705–1.023	<LOD-0.166
	0.509	0.873	0.216	0.367	0.211	0.852	0.152
	0.505–0.604	0.736–1.078	0.189–0.239	0.340–0.452	<LOD-0.273	0.705–1.023	<LOD-0.166
Honeydew (Ho)	7.646 ± 12.383	0.287 ± 0.090	0.252 ± 0.024	0.249 ± 0.089	0.368 ± 0.472	0.166 ± 0.183	0.475 ± 0.493
	0.354–21.944	0.184–0.348	0.225–0.268	0.171–0.346	0.133–0.913	<LOD-0.317	<LOD-0.985
	0.639	0.329	0.264	0.230	0.107	<LOD	0.442
	0.354–21.944	0.184–0.348	0.225–0.268	0.171–0.346	0.133–0.913	<LOD-0.317	<LOD-0.985
Linden (L)	2.064 ± 0.278	0.200 ± 0.051	1.679 ± 0.338	0.212 ± 0.211	0.312 ± 0.080	1.085 ± 0.276	1.973 ± 2.142
	1.818–2.454	0.152–0.271	1.227–1.998	<LOD-0.403	0.240–0.402	0.726–1.399	<LOD-3.982
	1.993	0.188 * B	1.746 ** B	0.221 * B	0.304 * B	1.107 * L	1.954
	1.872–2.257	0.165–0.235	1.427–1.931	0.151–0.392	0.245–0.380	0.910–1.259	0.123–3.822
Multifloral dark (Md)	0.680 ± 0.258	1.572 ± 0.964	0.261 ± 0.075	0.631 ± 0.325	0.108 ± 0.047	<LOD	0.633 ± 1.164
	0.443–1.045	0.185–2.235	0.190–0.334	0.144–0.804	<LOD-0.146	<LOD	<LOD-2.376
	0.615	1.934	0.260	0.789	0.128	<LOD	0.087
	0.517–0.842	0.917–2.227	0.197–0.325	0.463–0.800	<LOD-0.136	<LOD	<LOD-1.267
Multifloral light (Ml)	0.646 ± 0.551	0.191 ± 0.061	0.332 ± 0.333	0.357 ± 0.185	0.108 ± 0.070	0.178 ± 0.396	1.741 ± 2.479
	0.193–1.596	0.108–0.251	<LOD-0.885	0.158–0.235	<LOD-0.165	<LOD-0.885	0.094–5.654
	0.541	0.179 ** B	0.225	0.373	0.118	<LOD	0.155
	0.354–0.545	0.167–0.251	0.203–0.346	0.235–0.377	<LOD-0.145	<LOD	<LOD -2.782

Av. ± SD
Min-Max
Med
Q1-Q3

Table 4. Cont.

Variety (Sign)	3,4-DHBA	4-HBA	CA	<i>p</i> -CA	VA	SA	<i>t</i> -FA
Nectar-honeydew (Nh)	1.019 ± 0.849	0.235 ± 0.068	0.512 ± 0.667	0.299 ± 0.280	0.116 ± 0.033	0.410 ± 0.541	2.773 ± 4.607
	0.233–2.222	0.140–0.298	<LOD–1.493	0.154–0.717	<LOD–0.189	<LOD–1.141	0.090–9.656
	0.810	0.251 * B	0.278	0.178	0.107	0.249	0.673 * B
	0.488–1.550	0.193–0.277	0.134–0.891	0.174–0.457	<LOD–0.152	<LOD–0.819	0.193–5.353
Rape (R)	0.455 ± 0.379	0.256 ± 0.139	0.334 ± 0.245	0.262 ± 0.145	0.102 ± 0.068	0.267 ± 0.313	0.124 ± 0.060
	<LOD–1.482	0.109–0.484	0.177–0.870	0.155–0.581	<LOD–0.246	<LOD–0.847	<LOD–0.237
	0.350 ** L, ** B	0.174 *** B	0.227	0.242 *** B	0.114	0.165	0.128
	0.265–0.385	0.153–0.411	0.199–0.237	0.168–0.325	<LOD–0.127	<LOD–0.419	0.096–0.155

3,4-DHEA—3,4-dihydroxybenzoic acid, 4-HBA—4-hydroxybenzoic acid, <LOD—below the detection limit, CA—caffeic acid, *p*-CA—*p*-coumaric acid, SA—syringic acid, *t*-FA—*trans*-ferulic acid, VA—vanillic acid. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3.4. Correlations

The analysis of the correlation (Table 5) between the content of phenolic compounds in honey showed a strong relationship between the content of 4-HBA and *p*-CA ($r = 0.82$, $p < 0.000$), between VA and SA ($r = 0.60$, $p < 0.001$), and between SA and CA ($r = 0.51$, $p < 0.000$). Among the remaining parameters, the correlation between color intensity and TPC ($r = 0.90$, $p < 0.001$), and color in Pfund scale and color intensity ($r = 0.82$, $p < 0.001$) should be emphasized. Additionally, it is worth noting the correlation between color intensity and 4-HBA ($r = 0.84$, $p < 0.000$).

Table 5. Correlations between individual parameters ($p < 0.05$).

Parameter 1	Parameter 2	r	p
Color in Pfund scale	Colour intensity	0.82	0.001
Color in Pfund scale	TPC	0.77	0.001
Color in Pfund scale	Diastase number	0.51	0.001
Color in Pfund scale	3,4-DHBA	0.75	0.001
Color in Pfund scale	SA	−0.33	0.021
Color in Pfund scale	<i>p</i> -CA	0.51	0.001
Color in Pfund scale	<i>t</i> -FA	−0.57	0.001
Color in Pfund scale	CA	−0.45	0.001
Colour intensity	TPC	0.90	0.001
Colour intensity	Diastase number	0.51	0.001
Colour intensity	Water	0.33	0.020
Colour intensity	4-HBA	0.84	0.001
Colour intensity	VA	−0.39	0.005
Colour intensity	SA	−0.45	0.001
Colour intensity	<i>p</i> -CA	0.68	0.001
Colour intensity	<i>t</i> -FA	−0.52	0.001
Colour intensity	CA	−0.46	0.001
DPPH	Water	−0.37	0.008
DPPH	<i>p</i> -CA	−0.35	0.01
DPPH	<i>t</i> -FA	0.45	0.001
TPC	Diastase number	0.58	0.001
TPC	3,4-DHBA	0.33	0.020
TPC	4-HBA	0.79	0.001
TPC	VA	−0.30	0.038
TPC	<i>p</i> -CA	0.60	0.001
TPC	<i>t</i> -FA	−0.57	0.001
TPC	CA	−0.31	0.001
Diastase number	4-HBA	0.56	0.001
Diastase number	<i>p</i> -CA	0.55	0.001
Water	Electrical conductivity	−0.37	0.009
Water	4-HBA	0.31	0.026
Water	VA	−0.37	0.009
Water	<i>p</i> -CA	0.32	0.023
Water	CA	−0.36	0.011
Electrical conductivity	3,4-DHBA	0.40	0.005
Electrical conductivity	VA	0.29	0.040
Electrical conductivity	CA	0.42	0.002
FRAP	Colour in Pfund scale	0.68	0.001
FRAP	Colour intensity	0.82	0.001
FRAP	TPC	0.82	0.001
FRAP	Diastase number	0.50	0.001
FRAP	Electrical conductivity	0.38	0.008
FRAP	3,4-DHBA	0.53	0.001
FRAP	4-HBA	0.73	0.001

Table 5. Cont.

Parameter 1	Parameter 2	r	p
FRAP	<i>p</i> -CA	0.58	0.001
FRAP	<i>t</i> -FA	−0.38	0.006
3,4-DHBA	SA	0.30	0.034
3,4-DHBA	CA	0.37	0.009
4-HBA	SA	−0.29	0.040
4-HBA	<i>p</i> -CA	0.82	0.001
4-HBA	<i>t</i> -FA	−0.46	0.001
4-HBA	CA	−0.36	0.011
VA	SA	0.60	0.000
VA	<i>p</i> -CA	−0.32	0.024
VA	CA	0.60	0.001
SA	<i>p</i> -CA	−0.31	0.028
SA	CA	0.51	0.001
<i>p</i> -CA	<i>t</i> -FA	−0.30	0.038
<i>p</i> -CA	CA	−0.29	0.040
<i>t</i> -FA	CA	0.47	0.001

3,4-DHEA—3,4-dihydroxybenzoic acid, 4-HBA—4-hydroxybenzoic acid, CA—caffeic acid, *p*-CA—*p*-coumaric acid, SA—syringic acid, *t*-FA—trans-ferulic acid, TPC—total phenolic content, VA—vanillic acid.

It should be emphasized that we noted a positive correlation between the % of free radical scavenging in DPPH assay and the *t*-FA content ($r = 0.45$, $p < 0.001$).

3.5. Chemometric Analyzes

Cluster analysis performed for variables showed groups that are similar. One group was *p*-CA and 4-HBA, while the other group was *t*-FA, CA, SA, VA, and 3,4-DHBA (Figure 3).

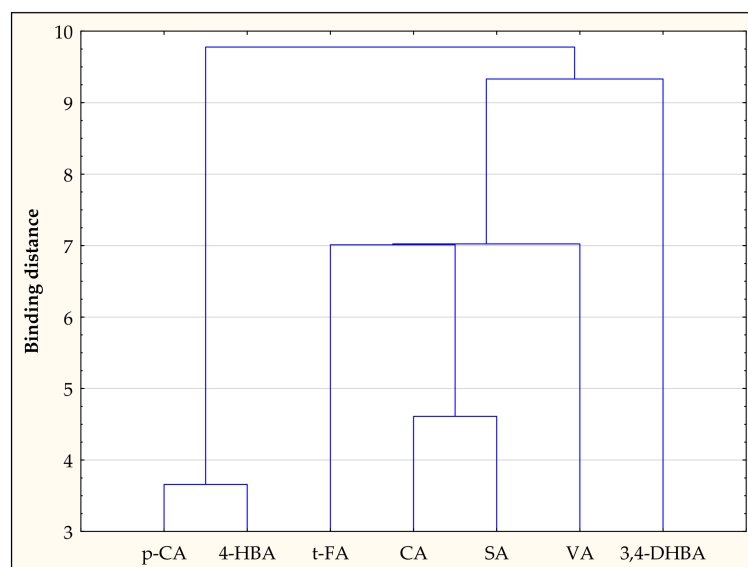


Figure 3. Cluster analysis for variables.

The analysis carried out for the cases, based on the contents of phenolic acids, mainly distinguished honeydew honey. The focus on linden honey is also worth emphasizing. Multiflower dark honeys have also qualified for the group containing buckwheat honey (Figure 4).

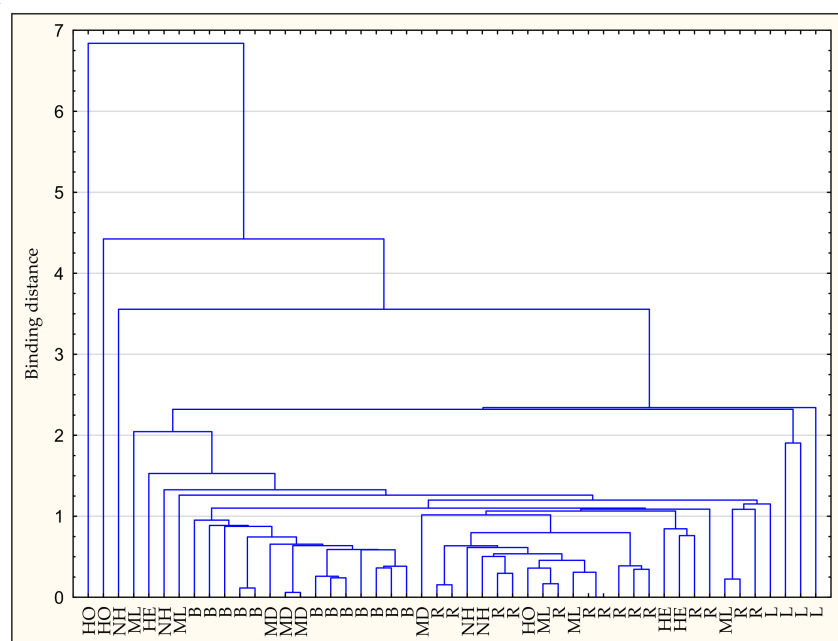


Figure 4. Cluster analysis for cases. B—buckwheat honey, HE—heather honey, HO—honeydew honey, L—linden honey, MD—multifloral dark honey, ML—multifloral light honey, NH—nectar-honeydew honey, R—rape honey.

Then, principal components analysis (PCA) was carried out, the purpose of which was to reduce the variables and classify the honey varieties. The first main component accounted for 43.84% of the variance; the second, 17.31%; the third, 14.45% (total 75.60%); and the subsequent components less than 10% of the variance.

On the basis of the eigenvectors, it can be assessed that factor 1 is related to the following components: *p*-CA (0.44), 4-HBA (0.41), SA (−0.46), and CA (−0.47). The second component is related to 4-HBA (0.54) and *p*-CA (0.50), and the third component 3,4-DHBA (−0.79). Figure 5 shows 2W plots of factor coordinates of the variables. Points are significant factor loadings for individual components. The farther a given load is from the center of the circle, the greater the correlation of the variable with the factor axis. Figure 6 present 2W plots of cases depending on the phenolic acids.

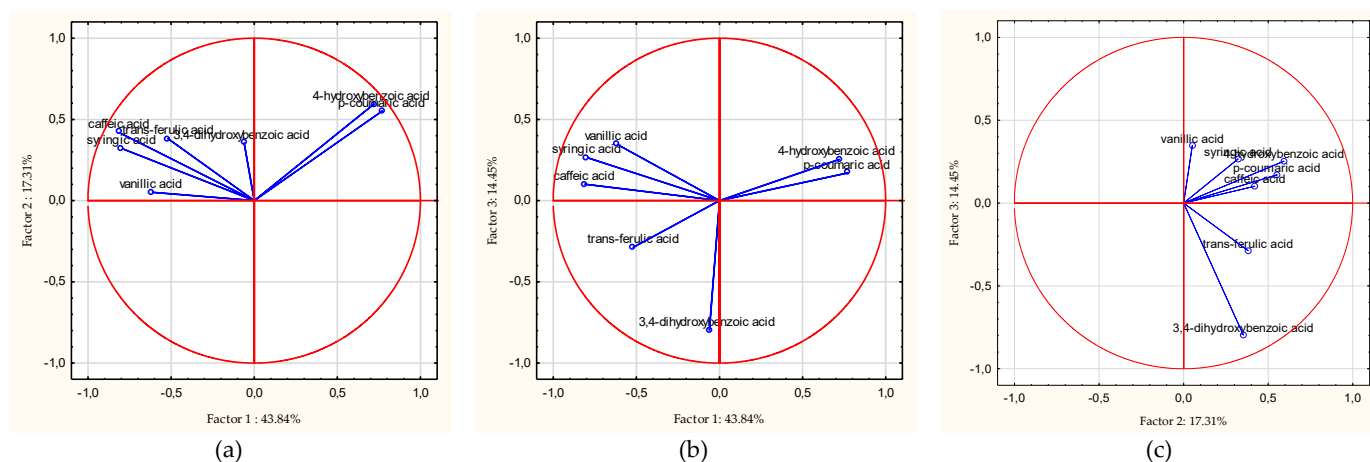


Figure 5. Projection of variables depending on the phenolic acids in a two – factor plane: factor 1 × factor 2 (a), factor 1 × factor 3 (b), factor 2 × factor 3 (c).

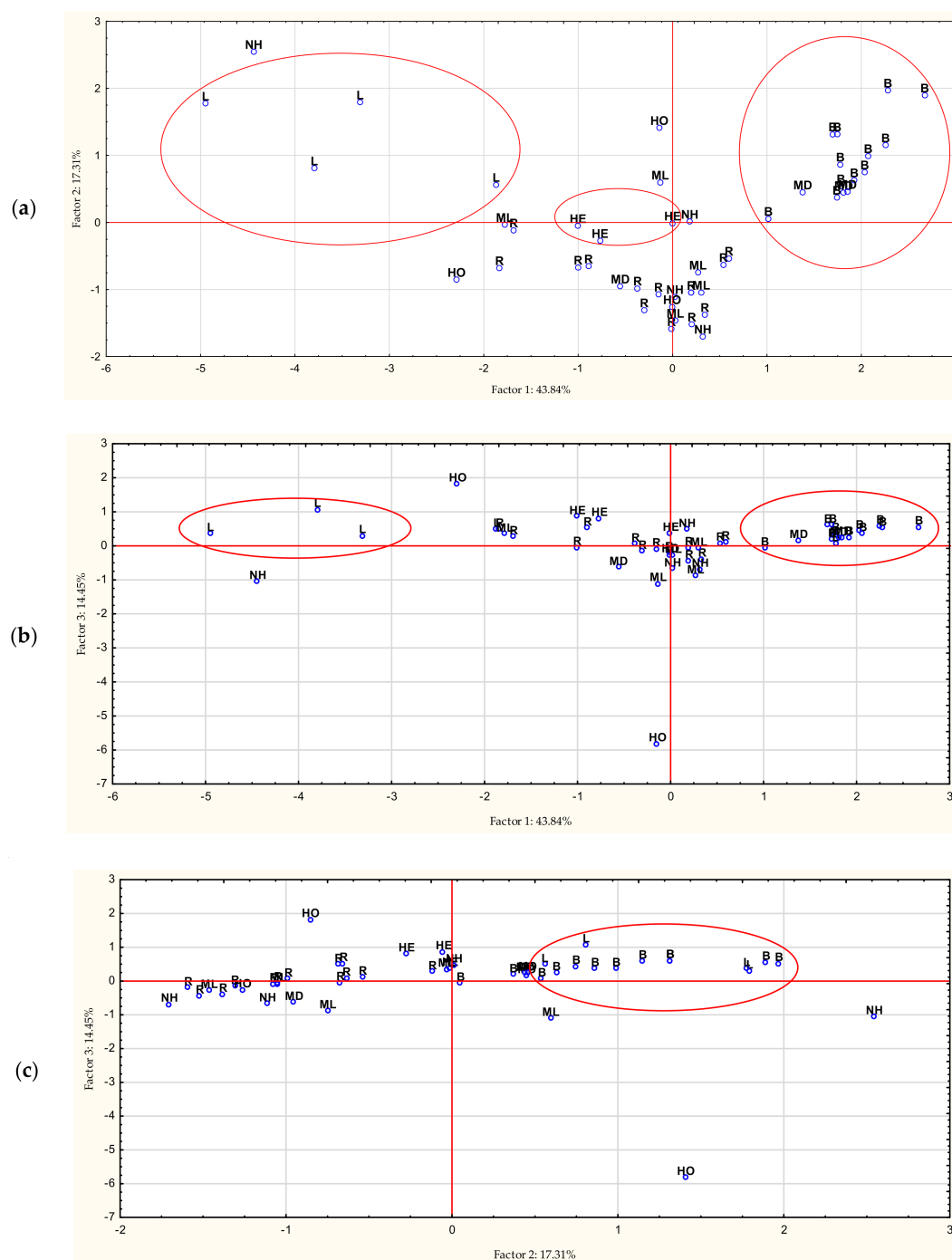


Figure 6. Projection of cases depending on the phenolic acids in a two – factor plane: factor 1 \times factor 2 (a), factor 1 \times factor 3 (b), factor 2 \times factor 3 (c).

4. Discussion

Natural bee honeys are characterized by a very rich composition, which determines their health-promoting properties. We have made an attempt to search for compounds that are characteristic of honey obtained in Poland. For rape, multifloral, nectar–honeydew, and honeydew honey, no characteristic phenolic compounds were found that could be considered determinants of the authenticity of these varieties.

Taking into account other quality criteria, honeydew honeys are distinguished by having the highest median of electrical conductivity ($1.181 \text{ mS} \times \text{cm}^{-1}$).

Multifloral honeys are characterized by a complex composition, without the dominant presence of one plant, which may result in the lack of advantage of a specific phenolic acid.

In addition, for rape honey, it may be necessary to establish a method with other acids. Moreover, rape honeys show the lowest electrical conductivity—the median was $0.242 \text{ mS} \times \text{cm}^{-1}$.

Buckwheat honeys from Poland show the darkest color, which results in the highest color value on the Pfund scale (median: 159.8 mm Pfund), the highest color intensity (2109.2 mAU), and the highest total phenolic content (196.59 mg GAE/100 g). Moreover, in these honeys, we showed the highest medians of 4-HBA (3.129 mg/100 g) and *p*-CA (0.804 mg/100 g). The studies conducted by Starowicz et al. (2021) [21] showed a lower value of TPC in honey of this variety (average: 141.1 mg GAE/100 g), while our research allowed us to conclude that the average value of this parameter is $182.60 \pm 61.08 \text{ mg GAE/100 g}$.

Heather honeys were characterized by the highest median of one of the determined phenolic acids: SA (0.852 mg/100 g). Research by Ecem Bayram et al. (2020) [22] indicated that 3,4-DHBA is a characteristic compound for this variety of honey. SA was a relatively abundant compound (193.77 and 242.33 mg/L). TPC in this variety, in line with our results, was $91.78 \pm 4.25 \text{ mg GAE/100 g}$, while the results published by Starowicz et al. (2021) [21] indicated a much higher content, at the level of 159.2 mg GAE/100 g.

Linden honey, despite the low content of phenolic compounds (27.50 mg GAE/100 g), was surprisingly characterized by high contents of 3,4-DHBA (1.993 mg/100 g), CA (1.746 mg/100 g), SA (1.107 mg/100 g), VA (0.304 mg/100 g), and *t*-FA (1.954 mg/100 g). Dimitrova et al. (2007) [23] determined the content of, inter alia, phenolic acids in 49 honey samples. In the case of linden honey ($n = 4$), the authors provided only the maximum value of CA—this was 1.57 mg/kg. Our analysis showed about a 10 times higher content of this ingredient, at the level of $1.679 \pm 0.338 \text{ mg/100 g}$, and the maximum value was 1.998 mg/100 g. The research carried out on linden honey from Turkey showed a characteristically high CA content (642.94 mg/L) [22], which was consistent with our observations ($1.679 \pm 0.338 \text{ mg/100 g}$). The content of SA was indicated only as of the maximum value (0.29 mg/kg)—in our study, the average content was $1.085 \pm 0.276 \text{ mg/kg}$. The average VA content in honey of this variety was indicated by the authors at the level of 1.19 mg/kg, while our research indicated a value almost two times higher (0.312 mg/100 g). These results are slightly divergent due to the fact that the apiaries from which the honey was obtained differed in geographical location—in the case of Dimitrova et al. (2007) [23], these were honeys from producers from Denmark, France, Germany, Italy, the Netherlands, Portugal, United Kingdom, and Spain, while in our study, all honeys were from Poland. Our analyses also show the existence of many dependencies between the measured phenolic acids, as well as other quality parameters. A high positive correlation between the contents of VA and SA and between SA and CA may indicate the common presence of individual phenolic acids in nectar, which is particularly visible in the case of linden honey.

The content of phenolic acids such as 3,4-DHBA, 4-HBA, VA, SA, *p*-CA, FA, CA in buckwheat and heather honey from Poland can be compared with the results obtained by Jasicka-Misiak et al. in 2012 [24], including heather ($n = 15$) and buckwheat honey ($n = 7$). In this study, similar contents of 3,4-HBA were obtained: our research showed the content of this compound at the level of $1.403 \pm 0.419 \text{ mg/100 g}$, while Jasicka-Misiak et al. [24] showed a level of about 1.228 mg/100 g (average content based on the determination of seven samples). The average VA content found in our study was approximately 10 times lower than in the study published by Jasicka-Misiak et al. [24]. The content of CA and SA in heather and buckwheat honey is low, in some cases below the detection limit, which is confirmed by both our research and the above-mentioned team of authors. According to our determinations, the *p*-CA content in buckwheat honey ranged from 0.558 to 1.004 mg/100 g, while the results obtained by Jasicka-Misiak et al. [24] were more divergent and indicated contents of 0.026 to 4.551 mg/100 g, and their average was almost three times higher.

Buckwheat honey shows the highest value of the TPC parameter. Numerous scientific publications indicate their rich composition; they are characterized, among others, by the presence of many volatile compounds, such as the occurrence of i.a. isovaleric acid in honey of this variety [25].

Searching for biomarkers of honey varieties is a task that has been carried out for over a dozen years [26]. For example, Cabras et al. (1999) [27] showed that the marker for strawberry honey is 2,5-dihydroxyphenylacetic acid, called homogentisic acid. Its content is around 378 ± 92 mg/kg. On the other hand, studies characterizing heather honey from Poland showed the presence of a less common compound: 4-hydroxy-3-(1-methylethyl) benzaldehyde [28]. Lumichrome is indicated as a honey marker for Polish yellow sweet clover [29].

Literature data show that the phenolic acids contained in honey can penetrate lymphocytes and protect DNA from oxidative damage by scavenging hydrogen peroxide and chelating ferrous ions, as shown in studies on mice [30].

Single phenolic acids show very promising activities. For example, ferulic acid has been shown to have anti-inflammatory properties [31] and potential anti-cancer properties [32], protocatechuic acid has anti-viral properties [33], and *p*-coumaric antidiabetic and antihyperlipidemic properties [34]. The above examples show that bee honey, being a mixture of many compounds with antioxidant properties, may show multidirectional activity.

The research conducted by Wilczyńska et al. (2010) showed that buckwheat honeys can be characterized by up to 100.00% of free radical scavenging capacity. Heather honeys turned out to be even more effective—all tested samples were characterized by a result of 100%. The lowest capacity was recorded for acacia honeys—from 25.58 to 35.90%. In our study, the median for buckwheat honeys was 41.1%. Moreover, Wilczyńska et al. showed that, in buckwheat honeys, the highest value of TPC was recorded (180.07 mg GAE/100 g). Our research showed a median of 196.59, with the highest value being 241.87 mg GAE/100 g [35].

Another study published by Pentoś et al. (2020) aimed to compare selected antioxidant properties of honey from Poland with Manuka honey. It was shown that Manuka honey has a TPC value of 492.65 ± 1.32 mg GAE/100 g, while the honey from Poland with the highest value of this parameter was buckwheat honey (334.04 ± 1.26 mg GAE/100 g). The honey with the second-highest TPC value was heather honey (183.85 ± 1.27 mg GAE/100 g) [36].

Dzugań et al. (2017) assessed, inter alia, results obtained in the FRAP test by Polish honeys. The highest result was obtained by buckwheat honeys (3635.49 ± 1328.22 μ mol TE/kg), followed by honeydew (2153.37 ± 663.92 μ mol TE/kg), while the lowest result was found for rapeseed honeys (656.73 ± 119.40 TE/kg). Our results were presented in a different way, but the trend was similar—we obtained the following results: 0.402 ± 0.010 , 0.323 ± 0.017 , and 0.030 ± 0.012 μ mol Fe²⁺/mL, respectively [7]. The studies by Beretta et al. (2005) also confirm that buckwheat and honeydew honeys are characterized by one of the highest results (800.7–23.8 and 772.0–21.5 μ M) [16].

Our study has some limitations. We tested different amounts of honey samples belonging to a particular variety. This was due to the improper labeling of honey by beekeepers. Future research should be based on an even selection of the number of samples. It seems necessary to develop a method that will allow the determination of all phenolic acids present in honey—this will allow for the creation of detailed characteristics and the development of characteristic ranges of variability. Additionally, it seems necessary to characterize the varieties in terms of the content of individual flavonoids.

5. Conclusions

Phenolic acids can be considered markers of the authenticity of Polish honeybee varieties, in particular, syringic acid, vanillic acid, and coffee acid for linden honeys, *p*-coumaric acid and 4-hydroxybenzoic acid for buckwheat honeys, and vanillic acid for honeydew honeys. Moreover, buckwheat honeys show the highest median of the TPC parameter,

which indicates a high content of phenolic compounds in the honeys of this variety. This variety of honey can be recommended to enrich the diet with antioxidant ingredients.

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Publikacja H3

Article

Comparative Analysis of Antioxidant Properties of Honey from Poland, Italy, and Spain Based on the Declarations of Producers and Their Results of Melissopalinalogical Analysis

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Abstract: Natural bee honeys are commonly used by patients for nutritional, preventive, and curative purposes. Honey varieties produced in other countries, including Italy and Spain, are gaining popularity. The aim of the study was to evaluate selected antioxidant properties of honey, taking into account the declared and actual variety. The research material consisted of 105 honey samples, including honeys from Poland ($n = 50$), from Spain ($n = 35$), and from Italy ($n = 20$). The variety was determined by the melissopalinalogical method, and in the case of honeydew honeys, the electrical conductivity was measured. Total phenolic content (TPC), color intensity, color in Pfund scale, DPPH, and FRAP were assessed. Polish buckwheat honeys, with confirmed botanical origin, are characterized by the highest median of the TPC (213.05 mg GAE/100 g), the highest color intensity (1.138 mAU), and the highest value in the FRAP test (0.394 $\mu\text{M Fe}^{2+}/\text{mL}$). In conclusion, proper labeling of bee honeys is necessary so as not to mislead consumers, and buckwheat honeys from Poland can be recommended to patients for prophylactic purposes in order to provide antioxidants in the diet.

Keywords: DPPH; FRAP; total phenolic content; Pfund scale; color intensity



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1. Introduction

Statistical data show that in 2020 there were 2,967,000 hives in Spain, 1,766,000 in Poland, and 1,687,000 in Italy, which makes these three countries among the five European countries with the highest number of bee colonies. China is in first place in terms of honey production, and the production of honey in this country covers as much as 24% of world production. The European Union countries are second in the world (12%) [1].

Melissopalinalogy is a field of palynology whose aim is the quantitative and qualitative assessment of bee products in terms of pollen grains present in the microscopic specimen [2]. In the case of unifloral honeys, based on percentage pollen grains that are most dominant over other grains or reach the required level characteristic for the variety in the honey sediment, the variety of honey is named after the plant [3,4].

Consumers and patients, selecting varieties of honey, sometimes expect specific properties resulting from the variety. Food available for sale, in accordance with legal requirements, should be properly labeled. Among bee honeys, there is often the problem of incorrect determination of the type of honey by beekeepers, e.g., only on the basis of the color, texture, taste, smell, and observation of flowering and nectar secretion of plants. Data in the literature indicate numerous labeling irregularities. For example, in 2018, the

Office of Competition and Consumer Protection, operating in Poland, published the results regarding the evaluation of the quality of honeys. A total of 269 batches of honey were inspected. There were irregularities in the labeling of honeys in the case of linden honeys (the content of the main pollen from *Tilia* spp. was from 1.9 to 7.7%), buckwheat honey (the content of the main pollen from *Fagopyrum* Mill. in the range from 23.8 to 35.5%), dandelion honey (pollen content from *Taraxacum officinale* F.H. Wigg. was found to be 5.1%), and in acacia honey (the share of dominant pollen from *Robinia pseudoacacia* L. was found at the level of 22.2%) [5].

Due to the large number of samples of incorrectly labeled honeys and the time-consuming nature of the classic method, alternative methods are sought. An example is the automatic pollen analysis based on the image analysis technique. This method is based on the conversion of visual information into mathematical descriptions. For this purpose, inter alia, 2D and 3D morphological features, color, and texture are considered [6].

Data in the literature indicate promising antioxidant properties of honey, which can be used in the prophylaxis of many diseases. For example, Manuka honey from New Zealand has antioxidant, antiproliferative, and antibacterial properties, and can inhibit the process of carcinogenesis by influencing various molecular processes [7]. With the use of *Glioblastoma multiforme* U87MG cell cultures, it was shown that honeys from Poland are a promising factor with anti-proliferative and anti-metastatic properties [8]. Polyphenols present in honey contribute to the occurrence of overlapping mechanisms of chemopreventive action in multi-step carcinogenesis, including by inhibiting mutagenesis or inducing apoptosis. The antibacterial effect of honey is explained by, among other factors, content of flavonoids [9], defensin-1, and methylglyoxal. Another benefit involves a protective effect on the cardiovascular system [10].

Due to the growing interest of people in consuming honey for prophylactic and therapeutic purposes, as well as openness to products from other countries, the aim of the research was to evaluate selected antioxidant properties of honeys from Poland, Italy, and Spain, taking into account the variety declaration (provided by beekeepers) and the proper variety (the result of melissopalinalogical analyses).

2. Materials and Methods

2.1. Materials

The study included 105 samples of natural bee honeys available for sale in onsite and online stores in Poland. The research covered the most common bee honey varieties available for sale, from five different producers. Fifty honeys were obtained from apiaries in Poland, 35 in Spain, and 20 in Italy—five honeys of each variety. The names of the varieties were specified by beekeepers on the labels. Characteristics of the varieties are presented in Table 1.

Table 1. Characteristics of the analyzed honeys—breakdown in accordance with the manufacturers' declaration.

The Origin of the Samples	Variety of Samples
Italy (<i>n</i> = 20)	chestnut (<i>n</i> = 5), eucalyptus (<i>n</i> = 5), lemon (<i>n</i> = 5), orange (<i>n</i> = 5)
Poland (<i>n</i> = 50)	acacia (<i>n</i> = 5), buckwheat (<i>n</i> = 5), dandelion (<i>n</i> = 5), heather (<i>n</i> = 5), honeydew coniferous (<i>n</i> = 5), honeydew deciduous (<i>n</i> = 5), linden (<i>n</i> = 5), phacelia (<i>n</i> = 5), rape (<i>n</i> = 5), raspberry (<i>n</i> = 5)
Spain (<i>n</i> = 35)	almond (<i>n</i> = 5), chestnut (<i>n</i> = 5), heath (<i>n</i> = 5), lavender (<i>n</i> = 5), orange (<i>n</i> = 5), rosemary (<i>n</i> = 5), thyme (<i>n</i> = 5)

2.2. Methods

2.2.1. Determination of Variety

The basis of the method was developed by Louveaux et al. [11]. In order to determine the honey variety, 10 g of honey (accuracy 0.001 g) were weighed into a Falcon conical

tube, and water at 50 °C was added. The resulting solution was centrifuged for 10 min (3000 rpm), then the supernatant was removed and the analytical steps were repeated. The solution was then withdrawn with a pipette, leaving a suitable layer of liquid above the sediment, and mixed until a suspension was obtained, from which a microscope preparation was prepared. Then, using an optical microscope at 400 times magnification, at least 300 consecutive pollens were classified into botanical species, excluding pollen from nectarless and anemophilous plants. The obtained results were converted into percentages. At least two repetitions were made for each honey sample [12].

2.2.2. Determination of Water Content

The water content in bee honey corresponds to the refractive index, which is determined by the refractometric method. For this purpose, 5 g were weighed and melted in a water bath (Grant, A-BioTech, Wroclaw, Poland) at 40 °C. Then the honey was placed on the prism of the refractometer and the refractive index was read, and corrected for the ambient temperature. The water content is shown as % [12]. The determination of the water content was used to calculate the mass of honey that was dissolved to determine the electrical conductivity.

2.2.3. Determination of Electrical Conductivity

Then, based on the water content, the quantities of honey were calculated. Solutions were prepared which were brought to 20 °C and their electrical conductivity was measured. The result was then multiplied by the constant value, characteristic for the conductivity electrode. The results are expressed in mS/cm [12].

2.2.4. Determination of Total Phenolic Content

The determination of the total phenolic content (TPC) was carried out on the basis of the methodology developed by Zhou et al. [13], including our own modifications. Honey was weighed out (1 g), to which 9 mL of distilled water have been added. The dissolved sample was centrifuged for 5 min at 2000 rpm. Next, 0.25 mL of supernatant was taken, 1.25 mL of 0.2 N Folin–Ciocalteu reagent was added thereto, mixed for 5 min and 1 mL of Na₂CO₃ solution was added. Samples were kept in the dark for 120 min and the absorbance at 760 nm was measured against water, using the U-2001 spectrometer (Hitachi, Tokyo, Japan). Gallic acid solutions (Sigma-Aldrich, St. Louis, MO, USA) were used to obtain the calibration curve. The results were expressed as gallic acid equivalents (GAE) per 100 g.

2.2.5. Determination of Color Intensity (CI)

The principle of this method is based on the measurement of the color intensity, which comes from antioxidant components, including carotenoids and flavonoids [14].

In order to determine the color intensity, 5 g of honey was weighed (with an accuracy of 0.001 g), and water at 45 °C was added to the volume of 10 mL. The solutions were then sonicated in ultrasonic washer for 5 min and filtered through 0.45 µm syringe filters. In the next step, the absorbance of the solutions was measured against water at 450 and 720 nm using U-2001 spectrometer (Hitachi, Tokyo, Japan). Three determinations were made for each honey. The results are expressed in mAU [14].

2.2.6. Determination of Color in Pfund Scale (CP)

The determination consisted in weighing out 5 g of the sample (with an accuracy of 0.001 g) and dissolved up to 10 mL in distilled water. The solutions were placed in a water bath at 50 °C to dissolve the sugar crystals, and then the absorbance, against water, was measured at 635 nm using U-2001 spectrometer (Hitachi, Tokyo, Japan). The numerical determination of the color was calculated on the basis of the formula:

$$mm\ Pfund = -38.70 + 371.39 \times Abs,$$

where: *Abs*—is the absorbance value read [15].

2.2.7. Determination of Radical Scavenging Capacity by DPPH Assay (DPPH)

The anti-DPPH radical scavenging ability was tested according to the method described by Sánchez-Moreno et al. [16] with own modification.

The honey samples were dissolved in distilled water to obtain a concentration of 1 g/mL. Then 1800 mL of methanol DPPH solution (concentration 0.04 mg/mL) was added to 200 mL of honey solutions, the absorbance was measured at 517 nm. The samples were incubated for 30 minutes in the dark at room temperature, after which time the absorbance was measured again using a spectrophotometer (Hitachi, Tokyo, Japan). The percentage of free radical scavenging was calculated from the formula:

$$\text{DPPH [\%]} = \left[1 - \frac{Ax}{A0}\right] \times 100,$$

where Ax is the absorbance for the honey solution and $A0$ is the absorbance of the control (the honey solution before incubation).

2.2.8. Determination of FRAP

The FRAP test was performed according to the methodology described by Benzie and Strain [17], with our own modifications.

The FRAP reagent was prepared which contained 2.5 mL of a 10 mM TPTZ solution in 40 mM HCl, 2.5 mL of 20 mM FeCl_3 , and 25 mL of 0.3 M acetate buffer, pH 3.6. On 96-well plates, 20 μL of honey solution was mixed with 180 μL of FRAP reagent. The plates were incubated for 10 min at 37 °C and the absorbance of the reaction mixture was measured with a plate reader (UVM 340, Biogenet, Józefów, Poland) at 593 nm. The results are expressed as equivalent $\mu\text{moles of Fe}^{2+}/\text{mL}$ of sample.

2.3. Statistical Analysis

Statistical analyses of the obtained data were performed using the Statistica v.13.3 software (TIBCO Software Inc., Palo Alto, CA, USA). The normality of the data distribution was assessed by the Kolmogorov–Smirnov test, Lilliefors test and the Shapiro–Wilk test. P values < 0.05 were considered significantly different.

The relationship between continuous categorized data was assessed by standard ANOVA. For better data classification and overall parameter evaluation, chemometric analyses were performed, including cluster analysis and principal component analysis.

3. Results

Table 2 presents the results of the melissopalinalogical analysis. It was shown that among honeys from Italy, only samples of chestnut honeys were all characterized by the correct variety definition. In fact, all acacia, dandelion, and raspberry honeys from Poland should be given a different name of the variety. Of the Spanish honeys, only the chestnut and lavender honeys were correctly named. Overall, only 62% of the honey was correctly labeled by beekeepers.

Table 2. Results of the melissopalinalogical analysis.

Varieties Declared by Beekeepers	The Origin of the Samples	Percentage of Correctly Classified Samples	Percentage of Incorrectly Classified Samples
chestnut	Italy	100	0
eucalyptus		60	40
lemon		20	80
orange		40	60

Table 2. Cont.

Varieties Declared by Beekeepers	The Origin of the Samples	Percentage of Correctly Classified Samples	Percentage of Incorrectly Classified Samples
acacia	Poland	0	100
buckwheat		100	0
dandelion		0	100
heather		60	40
honeydew coniferous		100	0
honeydew deciduous		40	60
linden		80	20
phacelia		60	40
rape		100	0
raspberry		0	100
almond	Spain	20	80
chestnut		100	0
heath		80	20
lavender		100	0
orange		60	40
rosemary		80	20
thyme		80	20
TOTAL		62	38

Table 3 presents the results for individual determinations, taking into account the division into varieties according to the declaration on the packaging.

Table 3. The results of research on antioxidant properties—division into varieties according to the manufacturers' declaration.

Varieties Declared by Beekeepers (Sign)	Total Phenolic Content (mg GAE/100 g)	Color Intensity (mAU)	Color in Pfund Scale (mm Pfund)	DPPH (%)	FRAP (Equivalent μM of Fe^{2+} /mL of Sample)
Av. \pm SD Min–Max Med. Q1–Q3					
THE ORIGIN OF THE SAMPLES: ITALY					
chestnut (A)	95.1 \pm 16.7	0.346 \pm 0.241	121.8 \pm 12.98	63.5 \pm 3.4	0.222 \pm 0.030
	78.58–114.00	0.156–0.694	104.7–140.4	58.1–67.4	0.193–0.265
	87.4	0.192	123.1	64.4	0.216
	83.38–112.20	0.185–0.506	116.6–124.4	63.0–64.8	0.197–0.237
eucalyptus (B)	53.53 \pm 10.6	0.263 \pm 0.081	103.2 \pm 24.5	60.6 \pm 10.8	0.170 \pm 0.048
	47.64–72.41	0.176–0.376	80.6–142.2	43.2–71.0	0.118–0.214
	48.91	0.275	100.4	64.1	0.188
	47.99–50.73	0.194–0.292	84.8–107.8	57.7–66.8	0.119–0.210
lemon (C)	21.47 \pm 2.94	0.134 \pm 0.111	44.5 \pm 21.7	31.5 \pm 10.2	0.014 \pm 0.008
	18.01–24.34	0.051–0.323	31.7–83.1	15.2–42.1	0.006–0.0248
	21.89	0.12	35.1	32	0.011
	18.90–24.23	0.054–0.126	35.1–37.3	30.8–37.6	0.008–0.019
orange (D)	29.84 \pm 5.41	0.222 \pm 0.310	43.1 \pm 15.7	46.4 \pm 8.3	0.066 \pm 0.027
	22.89–36.04	0.080–0.776	33.5–71.1	35.4–57.4	0.047–0.113
	29.58	0.08	37	44.2	0.06
	26.49–34.21	0.075–0.108	35.8–38.1	44.2–51.5	0.051–0.059
TOTAL	49.99 \pm 30.83	0.241 \pm 0.206	78.1 \pm 40.0	50.5 \pm 15.3	0.118 \pm 0.089
	18.01–114.00	0.050–0.776	31.7–142.2	15.2–71.0	0.006–0.265
	41.84	0.18	81.8	54.4	0.115
	24.28–75.49	0.094–0.307	36.4–112.2	39.9–64.2	0.036–0.204

Table 3. Cont.

Varieties Declared by Beekeepers (Sign)	Total Phenolic Content (mg GAE/100 g)	Color Intensity (mAU)	Color in Pfund Scale (mm Pfund)	DPPH (%)	FRAP (Equivalent μM of Fe^{2+} /mL of Sample)
Av. \pm SD Min–Max Med. Q1–Q3					
THE ORIGIN OF THE SAMPLES: POLAND					
acacia (E)	21.29 \pm 5.97	0.043 \pm 0.014	21.9 \pm 21.7	34.2 \pm 16.0	0.026 \pm 0.023
	14.70–28.15	0.025–0.050	0.1–49.8	9.3–51.0	0.004–0.061
	24.04	0.045	25.3	37.1	0.026 *** E/F
	15.31–24.26	0.033–0.052	0.1–34.0	30.0–43.6	0.006–0.031
buckwheat (F)	212.63 \pm 37.71	1.421 \pm 0.724	248.2 \pm 63.98	39.1 \pm 15.0	0.391 \pm 0.014
	167.75–261.05	0.770–2.605	182.8–351.1	21.1–57.9	0.370–0.407
	213.05 *** C/F, ** D/F, *** E/F	1.138 *** E/F	237.3 ** C/F	43.5	0.394 *** C/F, * D/F
	184.95–236.35	1.015–1.578	212.2–257.8	26.6–46.3	0.383–0.400
dandelion (G)	46.84 \pm 13.85	0.206 \pm 0.118	133.8 \pm 47.6	49.0 \pm 16.0	0.125 \pm 0.059
	34.70–68.85	0.111–0.379	87.4–211.3	30.1–67.1	0.090–0.229
	40.75	0.133	127.9	53.1	0.1
	38.23–51.71	0.130–0.279	104.7–137.6	34.7–59.9	0.098–0.108
heather (H)	82.32 \pm 25.65	0.353 \pm 0.089	122.6 \pm 22.9	46.0 \pm 34.3	0.152 \pm 0.050
	49.42–116.60	0.265–0.471	90.2–149.6	6.8–100.0	0.118–0.238
	76.48	0.351	120.9	45	0.141
	71.72–97.37	0.271–0.406	113.9–138.3	30.3–47.9	0.120–0.142
honeydew coniferous (I)	98.38 \pm 23.03	0.517 \pm 0.151	244.8 \pm 171.8	54.8 \pm 20.5	0.276 \pm 0.079
	64.57–120.75	0.309–0.643	77.9–518.8	24.4–74.2	0.152–0.343
	109.35	0.61	244.8	61.1	0.312
	85.25–112.00	0.404–0.622	122.3–259.4	44.4–69.7	0.243–0.328
honeydew deciduous (J)	76.42 \pm 17.98	0.402 \pm 0.157	150.0 \pm 29.3	67.3 \pm 5.0	0.189 \pm 0.052
	45.58–92.41	0.237–0.559	126.4–200.1	59.3–71.5	0.107–0.242
	82.60 * C/J	0.424 * E/J	138.2	68.5	0.199 * C/J
	78.42–83.08	0.244–0.549	134.8–150.4	65.8–71.3	0.178–0.219
linden (K)	51.80 \pm 21.79	0.186 \pm 0.169	78.4 \pm 14.3	59.6 \pm 10.5	0.100 \pm 0.049
	36.61–89.66	0.098–0.489	61.0–100.8	51.0–76.7	0.074–0.188
	42.02	0.109	76.4	53.9	0.077
	40.04–50.70	0.108–0.128	75.7–78.7	53.8–62.9	0.077–0.082
phacelia (L)	42.97 \pm 28.67	0.158 \pm 0.207	76.3 \pm 61.3	61.5 \pm 24.3	0.098 \pm 0.084
	25.17–93.74	0.050–0.528	32.9–184.5	39.6–100.0	0.042–0.44
	30.96	0.06	55.6 *** C/L, ** G/L	50.2	0.057
	28.62–36.39	0.058–0.095	51.4–56.9	47.3–70.2	0.051–0.094
rape (M)	30.61 \pm 3.24	0.064 \pm 0.014	62.0 \pm 17.6	49.9 \pm 7.1	0.056 \pm 0.012
	27.28–35.96	0.050–0.080	44.4–85.3	43.0–58.1	0.043–0.075
	29.88 * F/M	0.063 ** F/M	61.7	48.7	0.056 ** F/M
	29.26–30.69	0.051–0.078	45.6–72.9	43.3–56.2	0.046–0.058
raspberry (N)	48.72 \pm 16.14	0.162 \pm 0.061	91.6 \pm 23.6	63.66 \pm 15.33	0.164 \pm 0.050
	34.13–73.20	0.107–0.246	70.1–126.7	45.95–79.39	0.118–0.240
	40.82	0.14	79.4	62.25	0.166
	38.64–56.85	0.113–0.204	77.2–104.7	51.74–78.97	0.121–0.173
TOTAL	71.20 \pm 56.32	0.351 \pm 0.453	122.9 \pm 92.8	52.5 \pm 19.5	0.157 \pm 0.114
	14.70–261.05	0.025–2.605	0.1–518.8	6.8–100.0	0.004–0.407
	50.06	0.22	102.7	51.4	0.119
	34.13–89.66	0.080–0.471	61.7–149.6	43.3–65.8	0.074–0.238

Table 3. Cont.

Varieties Declared by Beekeepers (Sign)	Total Phenolic Content (mg GAE/100 g)	Color Intensity (mAU)	Color in Pfund Scale (mm Pfund)	DPPH (%)	FRAP (Equivalent μM of Fe^{2+} /mL of Sample)
Av. \pm SD Min–Max Med. Q1–Q3					
THE ORIGIN OF THE SAMPLES: SPAIN					
almond (O)	68.14 \pm 13.89	0.290 \pm 0.113	138.4 \pm 22.7	67.0 \pm 6.57	0.149 \pm 0.050
	44.06–77.78	0.117–0.381	116.8–176.6	59.3–74.3	0.067–0.206
	73.98	0.336	131.5	65.8	0.158
	68.79–76.13	0.238–0.377	129.8–137.3	62.6–73.2	0.152–0.159
chestnut (P)	116.06 \pm 14.67	1.043 \pm 0.383	186.5 \pm 43.1	43.0 \pm 21.7	0.275 \pm 0.043
	100.14–129.95	0.665–1.441	136.8–236.7	10.5–66.9	0.218–0.317
	121.40 ** C/P, ** E/P	1.021 * C/P, *** E/P, ** M/P	198.4	40.3	0.288 * C/P, * D/P, * E/P
	100.61–128.20	0.667–1.423	147.1–213.5	39.2–58.3	0.243–0.308
heath (Q)	111.06 \pm 17.93	0.563 \pm 0.460	255.96 \pm 101.71	41.1 \pm 27.1	0.320 \pm 0.052
	88.59–131.25	0.045–0.957	137.8–403.69	8.8–63.5	0.244–0.367
	105.84 ** C/Q, * E/Q	0.851	251.6 * C/Q	57.73	0.348 ** C/Q, ** E/Q
	102.20–127.40	0.079–0.886	192.1–294.6	14.3–61.2	0.290–0.351
lavender (R)	53.23 \pm 7.95	0.238 \pm 0.111	130.3 \pm 39.8	60.4 \pm 13.9	0.154 \pm 0.041
	40.65–62.57	0.116–0.408	92.2–187.6	39.0–71.9	0.092–0.207
	54.73	0.213	113.1	68.1	0.159
	52.81–55.38	0.176–0.276	103.4–154.7	53.8–69.3	0.148–0.162
orange (S)	30.57 \pm 3.44	0.089 \pm 0.027	62.8 \pm 14.9	45.0 \pm 13.2	0.053 \pm 0.017
	27.84–34.48	0.071–0.136	42.2–84.2	21.9–53.6	0.034–0.071
	28.42 *** F/S	0.076 * F/S	62.5	51.5	0.049 ** F/S
	27.93–34.17	0.074–0.087	62.1–63.2	46.3–51.8	0.042–0.071
rosemary (T)	41.06 \pm 17.00	0.117 \pm 0.052	57.6 \pm 13.8	64.3 \pm 20.3	0.098 \pm 0.068
	23.22–59.64	0.051–0.179	40.7–78.3	38.2–84.9	0.015–0.165
	43.35	0.138	58.9	71	0.124 ** L/T, * Q/T
	24.01–55.10	0.078–0.142	50.8–59.5	47.9–79.4	0.015–0.165
thyme (U)	116.6 \pm 42.4	0.359 \pm 0.192	104.0 \pm 11.9	79.3 \pm 2.4	0.316 \pm 0.038
	92.2–190.8	0.195–0.673	90.7–118.2	75.6–81.8	0.276–0.358
	94.3 * C/U, * E/U	0.356	100.4 ** C/U	79.7 * C/U, * E/U	0.314 ** C/U, ** E/U
	92.66–112.95	0.210–0.364	96.1–114.5	78.8–80.7	0.281–0.352
TOTAL	76.67 \pm 39.58	0.386 \pm 0.382	133.6 \pm 78.0	57.2 \pm 20.6	0.195 \pm 0.110
	23.22–190.80	0.045–1.441	40.7–403.7	8.8–84.9	0.015–0.367
	73.98	0.213	116.8	61.2	0.165
	43.35–102.20	0.116–0.665	78.3–176.6	46.3–73.2	0.092–0.290

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, DPPH—method using 2,2-diphenyl-1-picryl-hydrazyl-hydrate.

Among honeys originating in Italy, chestnut honeys were characterized by the highest medians for most of the parameters tested: TPC (87.40 mg GAE/100 g), 123.1 mm Pfund, 64.4% free radical scavenging, and 0.216 μM of Fe^{2+} /mL sample. The highest median color intensity was found for eucalyptus honeys (0.275 mAU) (Table 3).

The analysis of selected antioxidant parameters of Polish honeys showed that buckwheat honeys were characterized by the highest medians for most parameters: 213.05 mg GAE/100 g, 1.138 mAU, and 0.394 μM of Fe^{2+} /mL sample. Among honeydew honeys, the highest color median was demonstrated for honeydew coniferous honeys (244.8 mm Pfund), and the highest free radical scavenging ability was found in honeydew deciduous honeys (68.5%) (Table 3).

The analysis of the antioxidant parameters of Spanish honeys did not show any unequivocal conclusions. Chestnut honeys were characterized by the highest median TPC (121.40 mg GAE/100 g), color intensity (1.021 mAU), while heath honeys were distin-

guished by a high color value on the Pfund scale (251.6 mm Pfund) and a high value in the FRAP test (0.348 μM of Fe^{2+} /mL sample). Thyme honeys showed the highest free radical scavenging ability (79.7%) (Table 3).

Statistical analyses showed the greatest differences between the medians for individual parameters in the case of honeys from Poland and Italy, e.g., between buckwheat and lemon honeys (FRAP, CP, TPC), between Polish honeys such as buckwheat and acacia honeys (FRAP, CI, TPC), and between Italian and Spanish honeys such as lemon and chestnut honeys (FRAP, CP, TPC), lemon and heath honey (FRAP, CP, TPC), and lemon and thyme (FRAP, CP, TPC, DPPH) (Table 3).

Table 4 presents the results for individual parameters, the division into varieties was used according to the performed melissopalinalogical analysis. Chestnut honeys were characterized by the highest TPC value (87.40 mg GAE/100 g), color on the Pfund scale (123.1 mm Pfund) and the highest result in the FRAP test (0.216 μM of Fe^{2+} /mL sample). The highest median color intensity (0.292 mAU) and the highest free radical scavenging ability (66.8%) were demonstrated for eucalyptus honeys.

Table 4. The results of research on antioxidant properties—division into varieties according to the results of melissopalinalogical analysis.

Varieties According to the Melissopalinalogical Analysis (Sign)	Total Phenolic Content (mg GAE/100 g)	Color Intensity (mAU)	Color in Pfund Scale (mm Pfund)	DPPH (%)	FRAP (Equivalent μmoles of Fe^{2+} /mL of Sample)
		Av. \pm SD Min–Max Med. Q1–Q3			
THE ORIGIN OF THE SAMPLES: ITALY					
chestnut, $n = 5$ (A)	95.1 \pm 16.7	0.346 \pm 0.241	121.8 \pm 12.98	63.5 \pm 3.4	0.222 \pm 0.030
	78.58–114.00	0.156–0.694	104.7–140.4	58.1–67.4	0.193–0.265
	87.4	0.192	123.1	64.4	0.216
	83.38–112.20	0.185–0.506	116.6–124.4	63.0–64.8	0.197–0.237
eucalyptus, $n = 3$ (B)	56.01 \pm 14.20	0.314 \pm 0.054	116.8 \pm 22.3	67.3 \pm 3.4	0.204 \pm 0.013
	47.64–72.41	0.275–0.376	100.4–142.2	64.1–71.0	0.188–0.214
	47.99	0.292	107.8	66.8	0.21
	47.64–72.41	0.275–0.376	100.4–142.2	64.1–71.0	0.188–0.214
lemon, $n = 1$ (C)	21.89	0.12	35.1	37.6	0.025
	-	-	-	-	-
	21.89	0.12	35.1	37.6	0.025
	-	-	-	-	-
<i>Lotus corniculatus</i> L., $n = 6$ (D)	22.47 \pm 3.33	0.233 \pm 0.285	43.3 \pm 19.6	36.0 \pm 12.9	0.024 \pm 0.020
	18.01–24.49	0.051–0.776	31.7–83.1	15.2–51.5	0.006–0.051
	23.56 *** A/D	0.099	36.4	37	0.015
	18.90–24.24	0.054–0.230	35.1–37.3	30.8–44.2	0.008–0.047
multifloral, $n = 1$ (E)	50.73	0.176	84.8	43.2	0.119
	-	-	-	-	-
	50.73	0.176	84.8	43.2	0.119
	-	-	-	-	-
orange, $n = 3$ (F)	33.28 \pm 3.33	0.088 \pm 0.018	47.5 \pm 20.5	45.6 \pm 11.0	0.077 \pm 0.031
	29.58–36.04	0.075–0.108	33.5–71.1	35.4–57.3	0.006–0.113
	34.21 * A/F	0.080 * A/F	38.1	44.2	0.059
	29.58–36.04	0.075–0.108	33.5–71.1	35.4–57.3	0.006–0.113

Table 4. Cont.

Varieties According to the Melissopalinalogical Analysis (Sign)	Total Phenolic Content (mg GAE/100 g)	Color Intensity (mAU)	Color in Pfund Scale (mm Pfund)	DPPH (%)	FRAP (Equivalent μ moles of Fe^{2+} /mL of Sample)
		Av. \pm SD Min–Max Med. Q1–Q3			
other, $n = 1$ (G)	48.91	0.194	80.6	57.7	0.118
	-	-	-	-	-
	48.91	0.194	80.6	57.7	0.118
	-	-	-	-	-
TOTAL	49.99 \pm 30.83	0.241 \pm 0.206	78.1 \pm 40.0	50.5 \pm 15.3	0.118 \pm 0.089
	18.01–114.00	0.050–0.776	31.7–142.2	15.2–71.0	0.006–0.265
	41.84	0.18	81.8	54.4	0.115
	24.28–75.49	0.094–0.307	36.4–112.2	39.9–64.2	0.036–0.204
THE ORIGIN OF THE SAMPLES: POLAND					
buckwheat, $n = 5$ (H)	212.63 \pm 37.71	1.421 \pm 0.724	248.2 \pm 64	39.1 \pm 15.0	0.391 \pm 0.014
	167.75–261.05	0.770–2.605	182.8–351.1	21.1–57.9	0.370–0.407
	213.05 *** <i>D/H</i> , ** <i>F/H</i>	1.138 ** <i>D/H</i> , ** <i>F/H</i>	237.3	43.5	0.394 *** <i>D/H</i> , ** <i>F/H</i>
	184.95–236.35	1.015–1.578	212.2–257.8	26.6–46.3	0.383–0.400
heather, $n = 3$ (I)	80.83 \pm 33.80	0.314 \pm 0.080	126.0 \pm 31.6	33.3 \pm 22.9	0.133 \pm 0.014
	49.42–116.60	0.265–0.407	90.2–149.6	6.8–47.9	0.118–0.142
	76.48	0.271	138.3	45	0.141 ** <i>D/I</i>
	49.42–116.60	0.265–0.406	90.2–149.6	6.8–47.9	0.118–0.142
honeydew coniferous, $n = 5$ (J)	98.38 \pm 23.03	0.517 \pm 0.151	244.8 \pm 171.8	54.8 \pm 20.5	0.276 \pm 0.079
	64.57–120.75	0.309–0.643	77.9–518.8	24.4–74.2	0.152–0.343
	109.35 * <i>D/J</i>	0.61	244.8	61.1	0.312 * <i>D/J</i>
	85.25–112.00	0.404–0.622	122.3–259.4	44.4–69.7	0.243–0.328
honeydew deciduous, $n = 2$ (K)	87.50 \pm 6.94	0.486 \pm 0.089	163.2 \pm 52.1	70.0 \pm 2.2	0.221 \pm 0.031
	82.60–92.41	0.424–0.549	126.4–200.1	68.5–71.5	0.199–0.242
	87.5	0.486	163.2	70	0.221
	82.60–92.41	0.424–0.549	126.4–200.1	68.5–71.5	0.199–0.242
linden, $n = 4$ (L)	54.25 \pm 24.35	0.206 \pm 0.189	78.9 \pm 16.4	61.1 \pm 11.5	0.105 \pm 0.055
	36.61–89.66	0.098–0.489	61.0–100.8	51.0–76.7	0.074–0.188
	45.37	0.118	77	58.4	0.079
	38.32–70.18	0.103–0.308	68.3–89.5	52.4–69.8	0.075–0.135
multiflower, $n = 8$ (M)	56.70 \pm 32.38	0.250 \pm 0.190	92.3 \pm 64.7	54.0 \pm 29.7	0.130 \pm 0.101
	14.70–93.37	0.025–0.528	0.1–184.5	9.3–100.0	0.004–0.244
	58.65	0.242	109.3	56.6	0.113
	28.67–83.47.39	0.070–0.411	38.3–129.5	30.1–74.6	0.042–0.239
nectar-honeydew, $n = 2$ (N)	80.75 \pm 3.30	0.401 \pm 0.222	142.6 \pm 11.0	68.6 \pm 3.9	0.198 \pm 0.029
	78.42–83.08	0.244–0.559	134.8–150.4	65.8–71.3	0.178–0.219
	80.75	0.401	142.6	68.6	0.198
	78.42–83.08	0.244–0.559	134.8–150.4	65.8–71.3	0.178–0.219
phacelia, $n = 3$ (O)	42.97 \pm 28.67	0.158 \pm 0.207	76.3 \pm 61.3	61.5 \pm 24.3	0.098 \pm 0.084
	25.17–93.74	0.050–0.528	32.9–184.5	39.6–100.0	0.042–0.44
	30.96	0.060 * <i>H/O</i>	55.6 * <i>H/O</i>	50.2	0.057 * <i>H/O</i>
	28.62–36.39	0.058–0.095	51.4–56.9	47.3–70.2	0.051–0.094
rape, $n = 18$ (P)	36.95 \pm 11.79	0.118 \pm 0.089	75.1 \pm 32.5	53.6 \pm 16.7	0.094 \pm 0.054
	24.04–68.85	0.045–0.379	25.3–137.6	30.1–100.0	0.026–0.229
	34.41 ** <i>A/P</i> , *** <i>H/P</i>	0.094 ** <i>A/P</i> , * <i>H/P</i>	72	51.4	0.092 * <i>A/P</i> , ** <i>H/P</i>
	29.26–40.75	0.060–0.133	49.8–87.4	43.3–59.9	0.056–0.118

Table 4. Cont.

Varieties According to the Melissopalinalinological Analysis (Sign)	Total Phenolic Content (mg GAE/100 g)	Color Intensity (mAU)	Color in Pfund Scale (mm Pfund)	DPPH (%)	FRAP (Equivalent μ moles of Fe^{2+} /mL of Sample)
		Av. \pm SD Min–Max Med. Q1–Q3			
TOTAL	71.20 \pm 56.32	0.351 \pm 0.453	122.9 \pm 92.8	52.5 \pm 19.5	0.157 \pm 0.114
	14.70–261.05	0.025–2.605	0.1–518.8	6.8–100.0	0.004–0.407
	50.06	0.22	102.7	51.4	0.119
	34.13–89.66	0.080–0.471	61.7–149.6	43.3–65.8	0.074–0.238
THE ORIGIN OF THE SAMPLES: SPAIN					
almond, $n = 1$ (Q)	68.8	0.377	131.5	62.6	0.159
	-	-	-	-	-
	68.8	0.377	131.5	62.6	0.159
	-	-	-	-	-
chestnut, $n = 5$ (R)	68.14 \pm 13.89	0.290 \pm 0.113	138.4 \pm 22.7	67.0 \pm 6.57	0.149 \pm 0.050
	44.06–77.78	0.117–0.381	116.8–176.6	59.3–74.3	0.067–0.206
	73.98	0.336	131.5	65.8	0.158
	68.79–76.13	0.238–0.377	129.8–137.3	62.6–73.2	0.152–0.159
heath, $n = 4$ (S)	106.01 \pm 16.08	0.465 \pm 0.466	257.0 \pm 117.4	37.0 \pm 29.4	0.312 \pm 0.056
	88.59–127.40	0.045–0.886	137.8–403.7	8.8–63.5	0.244–0.367
	104.02 * D/S	0.465	243.3 * D/S	37.7	0.319
	95.40–116.52	0.062–0.868	164.9–349.1	11.6–62.4	0.267–0.357
lavender, $n = 5$ (T)	53.23 \pm 7.95	0.238 \pm 0.111	130.3 \pm 39.8	60.4 \pm 13.9	0.154 \pm 0.041
	40.65–62.57	0.116–0.408	92.2–187.6	39.0–71.9	0.092–0.207
	54.73	0.213	113.1	68.1	0.159
	52.81–55.38	0.176–0.276	103.4–154.7	53.8–69.3	0.148–0.162
multifloral, $n = 9$ (U)	70.39 \pm 34.72	0.290 \pm 0.269	118.6 \pm 65.9	65.4 \pm 11.8	0.172 \pm 0.115
	27.84–131.25	0.076–0.957	42.2–251.6	46.3–79.4	0.041–0.352
	73.98	0.195	116.8	65.8	0.152
	44.06–77.78	0.136–0.336	63.2–137.3	57.7–74.3	0.071–0.206
orange, $n = 3$ (V)	30.17 \pm 3.47	0.077 \pm 0.009	69.6 \pm 12.7	41.8 \pm 17.2	0.051 \pm 0.018
	27.93–34.17	0.071–0.087	62.2–84.2	21.9–51.8	0.034–0.071
	28.42	0.074 ** V/P	62.5	51.5	0.049
	27.93–34.17	0.071–0.087	62.1–84.2	21.9–51.8	0.034–0.071
rosemary, $n = 4$ (W)	37.55 \pm 17.42	0.102 \pm 0.045	57.3 \pm 16.0	60.49 \pm 21.3	0.086 \pm 0.070
	23.22–59.64	0.051–0.142	40.7–78.3	38.2–84.9	0.015–0.165
	33.68	0.108	55.1	59.5	0.081
	23.62–51.49	0.064–0.140	45.7–68.9	43.1–77.9	0.027–0.144
thyme, $n = 4$ (X)	117.49 \pm 48.88	0.400 \pm 0.195	107.3 \pm 10.7	79.4 \pm 2.7	0.307 \pm 0.038
	92.21–190.80	0.210–0.673	96.1–118.2	75.6–81.8	0.276–0.358
	93.48 * D/X	0.36	107.5	80.2 * D/X	0.298 ** D/X
	92.43–142.55	0.283–0.518	98.3–116.4	77.7–81.2	0.279–0.336
TOTAL	76.67 \pm 39.58	0.386 \pm 0.382	133.6 \pm 78.0	57.2 \pm 20.6	0.195 \pm 0.110
	23.22–190.80	0.045–1.441	40.7–403.7	8.8–84.9	0.015–0.367
	73.98	0.213	116.8	61.2	0.165
	43.35–102.20	0.116–0.665	78.3–176.6	46.3–73.2	0.092–0.290

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, DPPH—method using 2,2-diphenyl-1-picryl-hydrazyl-hydrate.

Among the honeys obtained from Poland, buckwheat honeys were characterized by the highest median for three parameters: TPC (213.05 mg GAE/100 g), color intensity (1.138 mAU), and the value obtained in the FRAP assay (0.394 μM of Fe^{2+} /mL sample). The highest median for the color parameter on the Pfund scale was characteristic for honeydew coniferous honeys (244.8 mm Pfund), and the highest free radical scavenging capacity was for nectar-honeydew honeys (68.6%).

Among the honeys that were purchased from Spain, in terms of antioxidant properties, the highest values for four out of five tested parameters were found for heath honeys: TPC (104.02 mg GAE/100 g), color intensity (0.465 mAU), color on the Pfund scale (243.3 mm Pfund), and the FRAP assay result (0.319 μM of Fe^{2+} /mL sample). Thyme honey was characterized by the highest scavenging capacity of free radicals (80.2%).

Further chemometric analyses were performed on the basis of the appropriate varieties, shown in the melissopalinalogical method.

On the basis of performed the cluster analysis we can observe that first cluster was mainly dark honeys, such as buckwheat, honeydew coniferous, or multifloral (Figure 1).

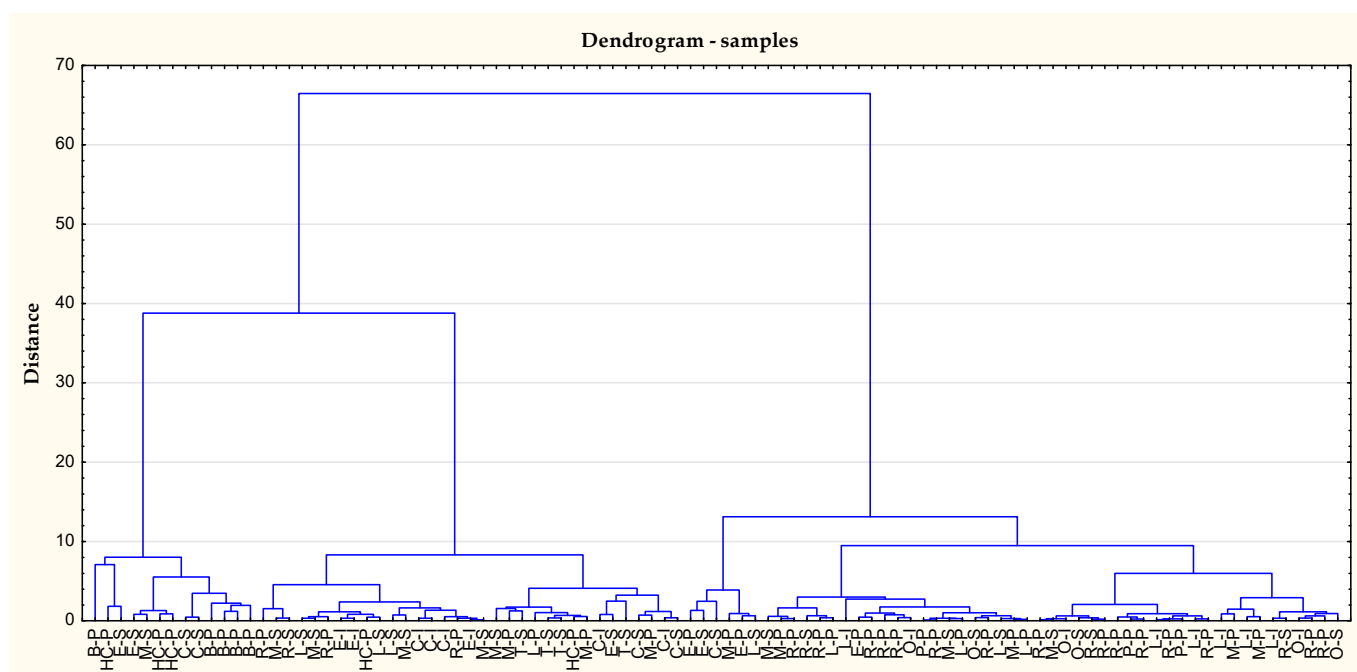


Figure 1. Dendrogram for the studied cases. B-P—buckwheat-Poland, C-I—chestnut-Italy, C-S—chestnut-Spain, E-I—eucalyptus-Italy, E-P—*Ericace*-Poland, E-S—*Erica*-Spain, HC-P—honeydew-Poland, L-I—*Lotus corniculatus* L.-Italy, L-P—lime-Poland, L-S—lavender-Spain, M-P—multifloral-Poland, M-P—multifloral-Poland, M-S—multifloral-Spain, O-I—orange-Italy, O-S—orange-Spain, P-P-phacelia-Poland, R-P—rape-Poland, R-S—rosemary-Spain, T-S—thyme-Spain.

Principal component analysis distinguished three main components. The first component accounted for 65.82% of the total variance, the second—21.89%, and the third—6.84%, which was a total of 94.55%.

Figure 2 presents a scatter plot of the assessed bee honey varieties in the space of two main components.

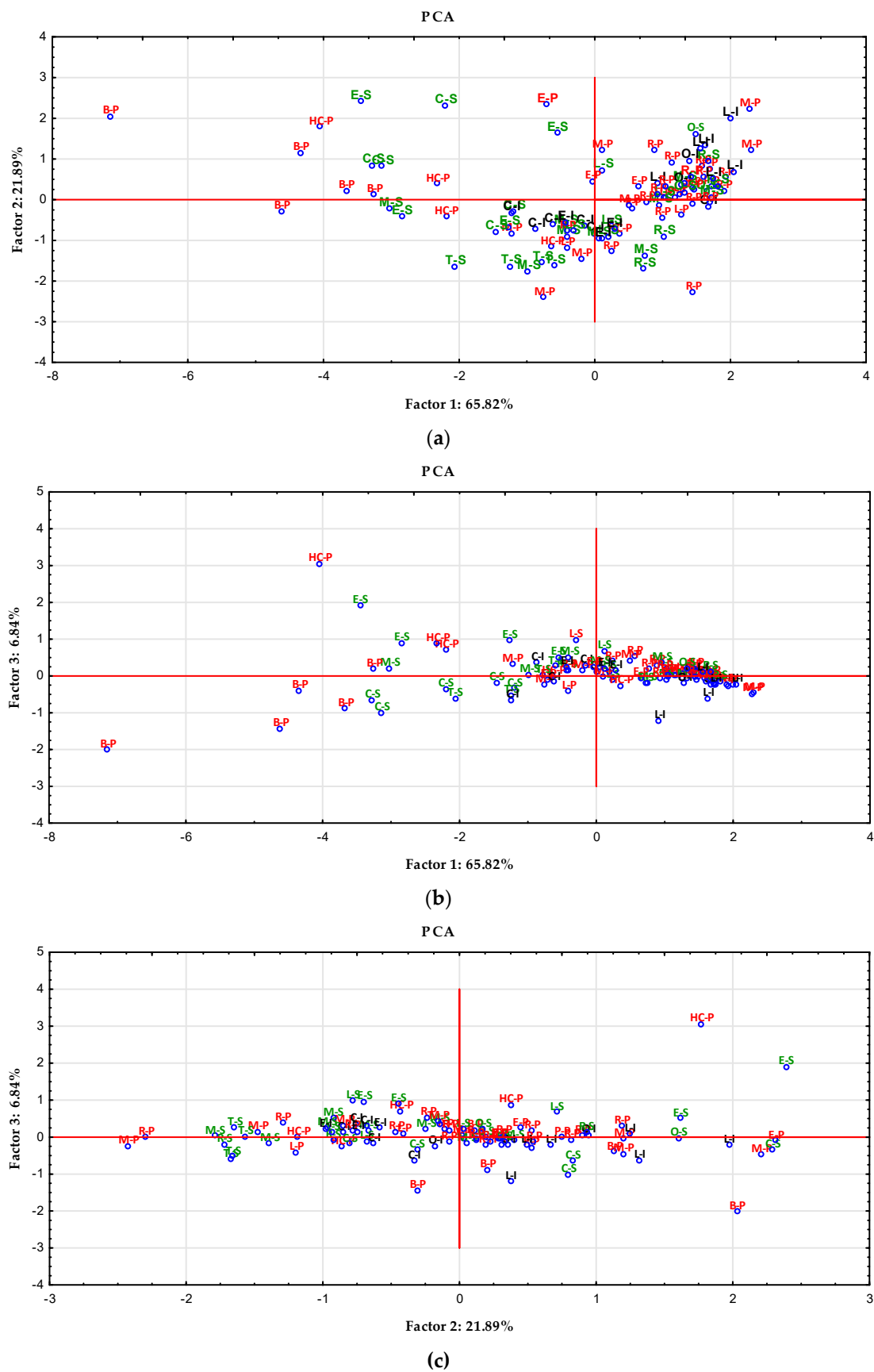


Figure 2. Projection of cases depending on the selected antioxidants parameters in a two-factor plane: factor 1 \times factor 2 (a), factor 1 \times factor 3 (b), factor 2 \times factor 3 (c).

DPPH showed practically no correlation with the first component (0.01), the correlation of the remaining four variables is negative, at a similar level (FRAP: -0.51 , TPC: -0.52 , CI: -0.49 , Pfund: -0.48). In the case of the second component, the strongest negative correlation was demonstrated with DPPH (-0.95), negative correlation was also shown by FRAP (-0.24), and TPC—practically no correlation (-0.06). The correlation with CI (0.14) and Pfund (0.15) is at a similar level. In the case of the third factor, the strongest positive correlation is seen with the Pfund factor (0.74). There is also a positive correlation between FRAP (0.16). No correlation (0.01) for DPPH. A stronger negative correlation was demonstrated for CI (-0.59) than for TPC (-0.28).

The comparison of antioxidant properties between honeys from Poland, Italy, and Spain shows no differences between these parameters—except for a significantly higher median color on the Pfund scale of honeys from Spain, compared to honeys from Italy (Table 5).

Table 5. The results of research on antioxidant properties—division into country of origin.

Origin (Sign)	Total Phenolic Content (mg GAE/100 g)	Color Intensity (mAU)	Color in Pfund Scale (mm Pfund)	DPPH (%)	FRAP (Equivalent μM of Fe^{2+} /mL of Sample)
Italy (A)	51.66 ± 32.81	0.255 ± 0.221	80.1 ± 42.2	51.3 ± 16.1	0.123 ± 0.094
	18.01–114.0	0.051–0.776	31.7–142.2	15.2–71.0	0.006–0.265
	36.04	0.184	83.1	57.3	0.113
	24.34–78.58	0.080–0.323	37.0–116.6	42.1–64.4	0.047–0.210
Poland (B)	70.08 ± 58.61	0.343 ± 0.470	117.3 ± 95.3	51.0 ± 19.7	0.153 ± 0.118
	14.70–261.05	0.025–2.605	0.1–518.8	6.8–100.0	0.004–0.407
	43.8	0.136	86.4	50.6	0.113
	30.96–89.66	0.078–0.406	61.0–138.2	43.0–61.1	0.061–0.238
Spain (C)	76.90 ± 40.15	0.386 ± 0.388	133.7 ± 79.1	57.1 ± 20.9	0.196 ± 0.111
	23.22–190.00	0.045–1.441	40.7–403.7	8.8–84.9	0.015–0.367
	75.05	0.211	115.7 * A/C	60.3	0.186
	43.35–102.20	0.116–0.665	78.3–176.6	46.3–73.2	0.092–0.290

* $p < 0.05$, DPPH—method using 2,2-diphenyl-1-picryl-hydrazyl-hydrate.

4. Discussion

The principle of determining the variety of honey is the method based on the assessment of the percentage of pollen grains of various plant species present in the honey sediment. The variety of honey is given the name of the plant on the basis of whichever grains are predominantly present [12]. The performance of these determinations is necessary to qualify the honey samples to the varieties, and therefore constitutes the basis for inference about properties, including antioxidant properties. Incorrect labeling may mislead consumers, and in the case of scientific research, result in incorrect formulation of conclusions as to the properties of the varieties. Before starting the analyses, many authors obtain certificates of the authenticity of the variety from beekeepers [18]. At the same time, there are many varieties on sale, the names of which sometimes constitute an incentive to purchase rather than an indication of the actual variety.

The antioxidant properties of bee honeys are one of the compelling reasons why consumers or patients buy these bee products. There are many methods of determining the antioxidant properties of honey—the most common of them are: TPC, DPPH, and FRAP. Color intensity and color on the Pfund scale are parameters that may correlate with antioxidant properties.

TPC is one of the most frequently performed assays to evaluate the total content of phenolic compounds in food samples. Research conducted on buckwheat honeys from Poland by Haladarga et al. (2020) [18] showed almost 10 times lower TPC value than our analyses: 22.33 ± 0.81 and 17.95 ± 1.57 mg GAE/100 g vs. 212.63 ± 37.71 mg GAE/100 g. It should be emphasized that despite this fact, buckwheat honeys assessed by the above authors were characterized by the highest value of the tested parameter.

The DPPH test is based on the measurement of the free radical scavenging activity of a sample. This is observed as a decrease in the absorbance of the methanolic DPPH solution at 517 nm [19]. The research conducted by Wilczyńska et al. (2010) [20] was aimed at assessing the antioxidant properties of honeys from Poland. One of the parameters assessed was the ability to scavenge free radicals. Out of three samples of buckwheat honeys, one honey showed 100% free radical scavenging. Two more samples showed the values 68.88% and 56.39%, respectively. The honeys tested in our publication showed the ability to scavenge free radicals from 21.1% to 57.9%. Surprisingly, 100% free radical scavenging capacity was demonstrated in the case of multiflorous, phacelia, and rapeseed honeys.

One of the important parameters for the assessment of antioxidant properties is FRAP. This test is based on the direct measurement of antioxidants in a sample. It is based on the ability of the sample to reduce $\text{Fe}^{3+}/\text{Fe}^{2+}$ [19]. The reduction power of three varieties of honey was studied by Alzahrani et al. (2012) [21]. They showed that acacia honey has the highest potency (1.366 ± 0.006), and that it was higher than manuka honey (1.2106 ± 0.005). Our research also covered acacia honey from Poland—the name of the variety was based on the declaration of beekeepers. Interestingly, honeys of this variety showed approximately 15 times lower reduction power than buckwheat honeys (Table 2).

One way to determine color is the Pfund scale. The term color on the Pfund scale was introduced to classify color on a numerical scale or as categories. The latter method is based on assigning numerical results to each class: white water (0–8), extra white (9–17), white (18–34), extra light amber (35–50), light amber (51–85), amber (86–114), and dark amber (over 115) [15]. As these terms do not correspond directly to the nomenclature of honeys in Poland, we used universal numerical values in this publication. Data in the literature indicate a low color value in the case of acacia honeys (7.01 ± 8.27). Lime (71.79 ± 31.98) and rapeseed (86.91 ± 51.06) honeys are characterized by a much higher value of color. The color of honeydew honeys is much higher—at the level of 131.16 ± 51.64 mm Pfund [22]. Our analyses showed a slightly lower value for linden (61.1 ± 11.5) and rapeseed (53.6 ± 16.7) honeys. The color of honeydew honeys was the highest (244.8 ± 171.8), while buckwheat honeys (248.2 ± 64 mm Pfund) were characterized by slightly higher.

Another way to objectively assess the color of honey is to define it as the color intensity. The research conducted for buckwheat honeys by Beretta et al. (2006) [14] showed approximately 1.5 times higher color intensity than our research (2245 vs. 1421 mAU).

The antioxidant properties of bee honeys are the subject of research by many authors, due to the possibility of using their prophylactic properties in various disease entities. For example, Kishore et al. (2011) [23] compared the antioxidant properties of Tualang and other honeys in order to emphasize the beneficial properties of the former variety. Interestingly, the authors showed TPC for this variety at the level of 83.96 ± 4.53 mg GAE/100 g, while FRAP at the level of 121.89 ± 3.87 $\mu\text{M Fe}^{2+}$ /100 g. Buckwheat from Poland is characterized by almost three times higher total content of phenolic compounds (212.63 ± 5.97 mg GAE/100 g) and almost three times higher value obtained in the FRAP test (0.394 $\mu\text{M Fe}^{2+}$ /mL sample).

Buckwheat honeys are characterized by specific antioxidant properties. Interesting results were published by Deng et al. (2018). The authors compared the quality of buckwheat honeys from China and manuka honeys, the price of which is several times higher than that of other honey varieties. Buckwheat honey, as in our publication, was characterized by a high content of phenolic compounds (149.8 ± 3.7 mg GAE/100 g)—higher than manuka honey (56.1 ± 0.3 mg GAE/100 g) [24].

The influence of buckwheat honey consumption on antioxidant parameters in blood serum was assessed, among others, by Gheldof et al. (2003) [25]. Men ($n = 25$) consumed: 500 mL of water (control), water with buckwheat honey (160 g/L), black tea, black tea with buckwheat honey (160 g/L), black tea with sugar analogue (160 g/L, 45% fructose + 35% glucose + 20% water). The antioxidant capacity of the serum was assessed using the ORAC test (the oxygen radical absorbance capacity)—ability to absorb oxygen radicals, the TBARS test (the thiobarbituric acid reactive substances), and the ex vivo susceptibility

test of serum lipoprotein to oxidation induced by Cu^{2+} . The authors demonstrated the effectiveness of consuming buckwheat honey in increasing the antioxidant capacity in the ORAC test by 7% ($p < 0.05$).

Data in the literature indicate a high correlation between the parameters determining the antioxidant properties of natural honey. For example, a very high positive correlation was found between TPC and FRAP ($R = 0.89$), as well as between TPC and DPPH ($R = 0.92$) [14].

PCA was used as a method of showing similarities for honey samples from Algeria. One of the parameters tested was the content of polyphenols. Nine principal components were obtained, explaining 72% of the variance. The authors obtained a grouping of honey samples from botanical origin, among others [24]. Our analyses, however, distinguished three main components, which explained as much as 94.55% of the variability.

Buckwheat honey is valued by consumers for its organoleptic properties, distinct taste, and aroma. Buckwheat honeys from Poland are characterized by a high proportion of main pollen grains, compared to buckwheat honeys from other countries. In this bee honey, various derivatives of phenolic compounds have been demonstrated: benzoic acid derivatives (protocatechuic acid and p-hydroxybenzoic acid), cinnamic acid derivatives (3-O-caffeoylquinic acid, 5-O-caffeoylquinic acid, coumaroyl hexoside, caffeic acid, 5-O-p-coumaroylquinic acid, p-coumaric acid, cinnamic acid, ferulic acid, benzyl caffeate, prenyl caffeate, cinnamyl caffeate), flavones (luteolin 6-C-hexoside, luteolin 8-C-hexoside, vitexin, luteolin, apigenin, chrysoeriol, tricetin, chrysin, acacetin), flavanols (quercetin 3-O-(6''-rhamnosyl)-hexoside, quercetin 3-O-galactoside, quercetin 3-O-rhamnoside, quercetin, quercetin 3-methyl ether, kaempferol, herbacetin 8-methyl ether, isorhamnetin, dimethyl quercetin, rhamnetin, galangin, kaempferide), flavanols (aromadendrin, pinobanksin 5-methyl ether, pinobanksin, pinobanksin 3-acetate, pinobanksin 3-butyrate, pinobanksin 3-pentanoate), and flavanone (pinocembrin) [26].

Our study has several limitations. We obtained various amounts of samples from individual countries. Future research should include the same number of samples from each variety and represent all the varieties sourced in a given country, in order to reach unambiguous conclusions. We did not have information from beekeepers as to the period of obtaining individual varieties. An important aspect that should be taken into account is the annual honey harvesting period, as the antioxidant parameters may change, even within a given variety, over the course of months. In addition, in our study, we considered several of the most popular methods of assessing antioxidant properties. In future research, the assessment of the properties of bee honeys should be extended to include ABTS test, and total flavonoids.

5. Conclusions

In summary, the analysis of antioxidant properties, taking into account the country of origin, did not show any differences in antioxidant properties between honeys obtained from Poland, Italy, and Spain. Origin has no effect on antioxidant capacity—the most important thing is the source of the nectar or honeydew from which the bee honey is made. The evaluation of the antioxidant properties of individual varieties showed that buckwheat honeys, which are botanically confirmed, are characterized by a high total content of phenolic compounds and a high ability to reduce iron compounds, confirmed in the FRAP test. Moreover, their color intensity, assessed spectrophotometrically, is the highest, compared to other honeys. These results can be the basis for the promotion of honey as a source of antioxidants.

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Publikacja H4

Article

Eating Habits during the COVID-19 Pandemic and the Level of Antibodies IgG and FRAP—Experiences of Polish School Staff: A Pilot Study

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Abstract: The coronavirus 19 (COVID-19) pandemic has brought many changes in terms of lifestyle, education, stress levels, and social contacts. The aim of our research was to evaluate changes in eating habits, physical activity, and selected lifestyle elements in a group of school staff, as well as their immune response to vaccination against COVID-19, and FRAP (ferric reducing antioxidant power) level. In total, 108 primary school teachers and other school staff with integration departments were included in the study. An original survey was conducted with the school staff. Of the study group, 45.4% chose to be vaccinated against COVID-19. In this group, the level of IgG antibodies was assessed, as well as the level of FRAP before vaccination, and after the first and second dose. An original questionnaire was also carried out. A decrease in physical activity and an increase in the time spent in front of the computer have been demonstrated, but a positive observation was a favorable change in most eating habits. After the second dose of vaccination, all subjects achieved the appropriate level of IgG antibodies (above 22 U/mL), with the maximum level recorded in 51%. There was also a significant increase in FRAP levels in the group after the first and second dose of the vaccine compared to the baseline level; an issue that requires further observation.

Keywords: antioxidants; Poland; lifestyle; diet



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1. Introduction

The COVID-19 pandemic, i.e., acute respiratory distress syndrome, began in December 2019 in the city of Wuhan in central China, and continues to date [1]. On 30 January 2020, the World Health Organization classified COVID-19 as a public health threat of international concern [2]. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a new type of virus from the coronavirus family. Infection occurs through direct or indirect contact with a sick person and their secretions, especially as a result of talking, coughing, and sneezing [1,3]. SARS-CoV-2 infection can be asymptomatic or accompanied by symptoms, including fever, headache and dizziness, runny nose, joint pain, characteristic loss of smell and taste, and disturbed consciousness [1,3,4]. Complications after passing the virus are varied and are still widely studied. People who have had a hard time of COVID-19 may experience shortness of breath and physical and mental weakness after leaving the hospital. It is not clear how long the complications may persist [5]. The pandemic has caused a number of changes in societies around the world in health, social, and economic life [6]. States have taken appropriate measures to minimize the spread of the virus, such as disinfecting hands, rooms, and equipment, as well as making it compulsory to wear masks [7].

The UK was the first Western country to introduce vaccination against SARS-CoV-2 [8]. The World Health Organization (WHO) has officially approved seven types of vaccines. Two contain viral mRNA, three are based on non-replicating viral vectors, and the other two contain inactivated viruses [9]. Some people are concerned about the introduced vaccines, and are withholding a decision to vaccinate [10]. This is due, *inter alia*, to the lack of confidence in vaccines and the fear of side effects [10,11]. By 21 October 2021, according to WHO, 6,655,399,359 vaccine doses have been administered [12]. It seems that vaccinations can actually have a positive effect on reducing the severity of the disease, which is especially important in the elderly and those with chronic diseases [13]. Vaccinations are designed to stimulate our immune system, to create immune memory, and thus alleviate the course of the disease [14]. According to a 2021 study, the most common symptom reported by 78% of respondents after the first dose of vaccination was pain at the vaccination site. Other symptoms include pain in the extremities (47% of respondents), and fatigue (30%). Malaise, headaches, increased body temperature, and pain in muscles and joints occurred much less frequently [15].

COVID-19 has increased the level of stress in societies around the world. The reasons are, *inter alia*, uncertainty about work, and the need to quickly adapt to the prevailing conditions [16–18]. Increasing the share of stress also translated into a change in eating habits. Studies have found that the pandemic and related quarantine resulted in higher food consumption, which, in turn, led to weight gain (up to 30%) [19]. An increase in the consumption of comfort food such as sweets and fast-food was observed [19,20]. Moreover, a decrease in physical activity was also noted [21,22]. On the other hand, in smokers, the frequency of smoking increased, and in people addicted to alcohol, there was a higher consumption [19]. These changes also adversely affect the body's resistance by generating oxidative stress. This seems to be particularly important in the prevailing pandemic [23,24]. The diet should include ingredients with antioxidant properties, such as polyphenols, antioxidant vitamins (vitamin C, β -carotene, vitamin E, and vitamin D), as well as minerals (zinc, selenium, copper, and manganese) [24,25]. It has been shown that a high content of these components has a positive effect on the antioxidant potential of the organism [26,27]. In the Prevention with Mediterranean Diet (PREDIMED) study, one-year dietary intervention in the form of the Mediterranean diet significantly increased the FRAP index, which assesses the non-enzymatic antioxidant capacity in the blood by reducing iron ions [28]. Antioxidants are present, among others, in vegetables, fruits, nuts, seeds, herbs, and legumes [29–31].

School employees are an important professional group, exposed to contact with many people, unlike, *inter alia*, administration employees who worked remotely more often. Moreover, these people constitute a model of behavior for their charges; therefore, their behavior may shape the nutritional and health behavior among the young generation.

Due to the fact that the COVID-19 pandemic has caused a number of negative effects, including deterioration in mental and physical health, there is a constant need to investigate this issue. Therefore, the main aim of the study was to assess the eating habits and selected lifestyle elements in a school staff group, and the secondary aim was to assess their immune response to vaccination against COVID-19, and changes in FRAP levels.

2. Materials and Methods

2.1. Ethical Approval

The study was conducted in accordance with the Helsinki Declaration and Good Clinical Practice, as well as was approved by the Ethics Committee of the Medical University of Białystok, Poland (approval numbers: APK.002.20.2021, date of approval: 28 January 2021). Informed consent was given by all participants of the study.

2.2. Study Design and Participants

In total, 114 school employees residing in the Podlaskie Voivodeship (Białystok, Poland) were invited to participate in this study. The study participants were employed in

a primary school with integration departments, in which disabled children were taught. Therefore, despite the pandemic and school closures, this school worked in a hybrid system. This means that the school worked alternately online and stationary.

The survey was conducted from February to May 2021, and included three periods (before vaccination, 10 and 11 February; two weeks after the first dose of vaccination, 24 March; and two weeks after the second dose of vaccination, 19 May). At the first visit, the participants completed the basic questionnaire (participants personally collected the questionnaires, and had the opportunity to get answers to all questions from the persons conducting the research), and blood was collected for testing (the blood was taken by a professional nurse through a venous puncture). Body weight and height were entered independently by the respondents. Out of 114 respondents, we obtained completed questionnaires from 108 people. The subjects who decided to vaccinate against COVID-19 (45.4%) were invited to the second and third period of the study. Blood samples were taken again during the second and third period of the study, and an additional questionnaire was used. FRAP and antibodies were tested before, and after the first and second dose of vaccination (Figure 1).

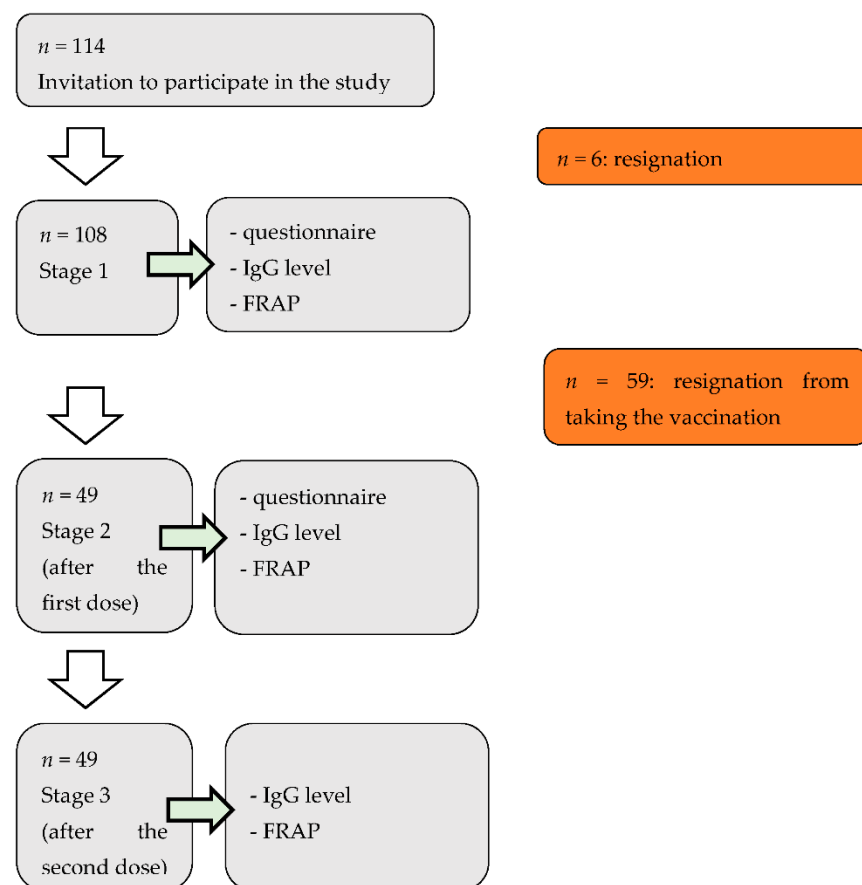


Figure 1. Study scheme. FRAP: ferric reducing antioxidant potential.

The key indicators in our study are: IgG level; FRAP level; and selected anthropometric, lifestyle, and vaccine response aspects. They are characterized below.

2.3. Applied Questionnaires

The basic questionnaire consisted of three parts: Part 1—questions about gender, age, height, weight, and weight changes during the COVID-19 pandemic, as well as type of work and seniority at school, current form of work (stationary, remotely), and own opinion on the topic of online learning during a pandemic; Part 2—questions about getting COVID-19, symptoms and complications, antibody levels, illness among household members,

being in quarantine, chronic diseases, and vaccination against COVID-19; Part 3—questions about changes in eating habits and lifestyle during the pandemic, including questions about experiencing stress, changes in hygiene habits, smoking, physical activity, changes in the consumption of different groups of products, the amount of food consumed per day, time spent in front of the computer, and hours of sleep (Table S1). An additional questionnaire included questions on the post-vaccination aspects: symptoms experienced and their duration (Table S2).

2.4. FRAP Assay

The total antioxidant potential of serum was measured spectrophotometrically using the FRAP (ferric reducing antioxidant power) method according to Benzie and Strain [32] on the spectrophotometer UV-Shimadzu (Shimadzu, Kyoto, Japan). This method is based on the reduction of the Fe^{3+} ions in the form of a complex with 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) to the Fe^{2+} ions. The TPTZ- Fe^{2+} complex has intense color with a maximum absorption at 593 nm wavelength. The intensity of color is directly proportional to the concentration of Fe^{2+} ions.

2.5. Antibodies Assay

The antibodies were measured in microplate reader (Rayto RT-6100C, Guangzhou, China) at 450 nm, using an immunoenzymatic kit for the determination of IgG antibodies against RBD (receptor-binding domain), Spike S1 protein subunit, and SARS-CoV-2 virus (COVID-19) in human serum or plasma (TestLine Clinical Diagnostics s.r.o., Brno, Czech Republic). The RBD specifically binds to the angiotensin-converting enzyme 2 (ACE2) of the host cell. The binding of RBD to ACE2 is highly associated with the formation of neutralizing antibodies.

The interpretation of the antibody level was as follows: lower than 18 U/mL is negative, 18 to 22 U/mL is borderline, and higher than 22 is positive. The maximum level that can be determined with this test is 400 U/mL.

Additionally, the Index of Positivity (IP) was calculated based on the following formula:

$$\text{IP} = \frac{\text{Absorbance of serum, plasma}}{\text{Mean absorbance of CUT-OFF}},$$

where: CUT-OFF—calibrator, 20 U/mL.

2.6. Statistical Analysis

Statistical analyses were performed using Statistica v. 13.3 (StatSoft, TIBCO Software Inc., Palo Alto, CA, USA). The results were considered statistically significant for $p < 0.05$. The Shapiro–Wilk, Kolmogorov–Smirnov, and Lilliefors tests were used to test the normality of the distribution of variables. The Mann–Whitney U test was used to compare groups without a normal distribution. A chi-squared test was used for variables expressed as categories. Correlations were calculated using the Spearman’s test.

3. Results

3.1. Characteristics of the Group

The first stage of our research involved 108 school employees, including 89 women and 19 men. The mean age of the study group was 46.3 ± 10.5 years, and the mean BMI was over 25 ($26.3 \pm 4.3 \text{ kg/m}^2$). The average professional experience of the respondents was 16.6 ± 12.1 years. The results are presented as the number of people (Table 1).

Table 1. Characteristics of the study group ($n = 108$).

Parameter	n	Av. \pm SD	Med.	Min.–Max	Q1–Q3
Gender (n , W/M)	89/19	-	-	-	-
Age (years)	108	46.3 \pm 10.5	48.5	24.0–70.0	39.5–54.0
Height (m)	108	168.0 \pm 6.6	168.0	153.0–190.0	163.0–170.5
Body weight (kg)	108	74.0 \pm 13.5	70.0	55.0–115.0	63.0–83.0
BMI (kg/m ²)	108	26.3 \pm 4.3	25.6	19.3–41.7	23.0–29.1
Work experience (years)	108	16.6 \pm 12.1	17.5	0.5–51.0	4.5–20.5

Av.—average, M—men, Max—maximum, Med.—median, Min.—minimum, Q1–Q3—quartile 1–quartile 3, SD—standard deviation, W—women.

Among those who reported an increase in their weight, the highest percentage of the studied group (39.0%) reported an increase in body weight by 3–5 kg. The largest percentage of the surveyed school employees conducted classes in grades 0–3 (37.0%) and 4–8 (30.6%). During the completion of the questionnaire, most of the respondents worked stationary (51.4%). The vast majority of respondents assessed that distance learning is worse than traditional education (85.4%) (Table 2).

Table 2. Baseline characteristic of study groups ($n = 108$).

Parameter	Total ($n = 108$) n (%)	Women ($n = 89$) n (%)	Men ($n = 19$) n (%)
Change in weight during a pandemic			
No change	51 (47.2)	41 (46.1)	10 (52.5)
It was increased in the range of 3–5 kg	42 (39.0)	36 (40.4)	6 (31.6)
It was increased in the range above 10 kg	5 (4.6)	4 (4.5)	1 (5.3)
It was reduced in the range of 3–5 kg	1 (0.9)	0 (0.0)	1 (5.3)
It was reduced in the range of 6–10 kg	8 (7.4)	7 (7.9)	1 (5.3)
It was reduced in the range above 10 kg	1 (0.9)	1 (1.1)	0 (0.0)
Type of work performed at school (multiple choice question)			
Teacher in grades 0–3	40 (37.0)	38 (42.7)	2 (10.5)
Teacher in grades 4–8	33 (30.6)	23 (25.8)	10 (52.6)
School administration	10 (9.3)	8 (9.0)	2 (10.5)
School service	29 (26.9)	24 (27.0)	5 (26.4)
How do you currently work?			
Stationary	55 (51.4)	48 (53.9)	7 (36.8)
Remotely	21 (18.7)	13 (14.6)	8 (42.1)
Stationary and remotely	32 (29.9)	28 (31.5)	4 (21.1)
How do you rate remote learning during a pandemic?			
Comparable to traditional teaching	16 (14.6)	14 (15.7)	2 (10.5)
Worse than traditional education	92 (85.4)	75 (84.3)	17 (89.5)

3.2. COVID Infection-Symptoms, Health Background

Of the study group, 21.3% of the respondents tested positive for COVID-19: all patients were female, but we did not show statistical significance ($p > 0.05$). Earlier, before our study, 7.4% of the study group had the level of IgG antibodies determined (Table 3).

Table 3. Experiences with COVID-19 among study group ($n = 108$).

Parameter	Total ($n = 108$)	Women ($n = 89$)	Men ($n = 19$)
	n (%)	n (%)	n (%)
Have you been tested positive for COVID-19?			
Yes	23 (21.3)	23 (25.8)	0 (0.0)
No	85 (78.7)	66 (74.2)	19 (100.0)
Have you had a COVID-19 antibody test performed?			
Yes	8 (7.4)	8 (9.0)	0 (0.0)
No	100 (92.6)	81 (91.0)	19 (100.0)
If you have had COVID-19, please mark the symptoms accompanying the disease (multiple choice question)			
Fever of 38 °C and above	11 (10.2)	10 (11.2)	1 (5.3)
Cough	10 (9.3)	9 (10.1)	1 (5.3)
Diarrhea	3 (2.8)	3 (3.4)	0 (0.0)
Nausea	4 (3.7)	4 (4.5)	0 (0.0)
Vomiting	1 (0.9)	1 (1.1)	0 (0.0)
Smell and taste disorders	16 (14.8)	15 (16.9)	0 (0.0)
Conjunctivitis	1 (0.9)	1 (1.1)	0 (0.0)
Difficulty breathing, difficulty drawing air	7 (6.5)	7 (7.9)	0 (0.0)
Muscle aches, fatigue	15 (13.9)	14 (15.7)	1 (5.3)
Other symptoms	7 (6.5)	7 (7.9)	0 (0.0)
Have any of your household members had a positive COVID-19 test?			
Yes	14 (13.0)	8 (9.0)	6 (31.6)
No	94 (87.0)	81 (91.0)	13 (68.4)
Were you in quarantine because of COVID-19?			
Yes	36 (33.3)	29 (32.6)	7 (36.8)
No	72 (66.7)	60 (67.4)	12 (63.2)
For what reason were you in quarantine? (38 answers)			
Own disease	6 (5.6)	6 (6.7)	0 (0.0)
Household disease	15 (13.9)	11 (12.4)	4 (21.1)
Co-worker disease	10 (9.3)	7 (7.9)	3 (15.8)
Return from abroad	2 (1.9)	2 (2.2)	0 (0.0)
Another	5 (4.6)	5 (5.6)	0 (0.0)
Do you suffer from chronic diseases?			
Yes	37 (34.3)	34 (38.2)	3 (15.8)
No	71 (65.7)	55 (61.8)	16 (84.2)
Have you been vaccinated against COVID-19?			
No	109 (100.0)	89 (100.0)	19 (100.0)
Yes	0 (0.0)	0 (0.0)	0 (0.0)
Would you report your willingness to be vaccinated against COVID-19 if it was possible?			
Yes	61 (56.5)	47 (52.8)	14 (73.7)
No	47 (43.5)	42 (47.2)	5 (26.3)
If not, why not? (30 answers)			
I don't believe vaccination is effective	3 (2.8)	2 (2.2)	1 (5.3)
I do not like the type of vaccine offered to the education staff	20 (18.5)	17 (19.1)	3 (15.8)
Other	7 (6.5)	7 (7.9)	0 (0.0)

Table 3. Cont.

Parameter	Total (n = 108)	Women (n = 89)	Men (n = 19)
	n (%)	n (%)	n (%)
If you have had COVID-19, do you think that your current health has returned to its pre-disease state? (23 answers)			
Yes	6 (5.6)	6 (6.7)	0 (0.0)
No	17 (15.7)	14 (15.7)	3 (15.8)
If you have suffered from COVID-19, what complications do you experience after the illness? (35 answers)			
General	7 (6.5)	7 (7.9)	0 (0.0)
From the respiratory system	5 (4.6)	5 (5.6)	0 (0.0)
From the cardiovascular system	6 (5.6)	6 (6.7)	0 (0.0)
Neurological and psychiatric	8 (7.4)	8 (9.0)	0 (0.0)
From the gastrointestinal tract	1 (0.9)	1 (1.1)	0 (0.0)
From the motor organ	3 (2.8)	3 (3.4)	0 (0.0)
From the sensory organs and the throat	5 (4.6)	4 (4.5)	1 (5.3)

COVID-19—coronavirus disease.

Respondents indicated that during COVID-19 infection, they mainly had the following symptoms: smell and taste disorders (14.8%), muscle aches and fatigue (13.9%), fever of 38 °C and above (10.2%), and cough (9.3%) In our study, 13% of respondents indicated that their household tested positive for COVID-19 (Table 3)

The most common reason for quarantine was illness of household members; this reason was indicated by 13.9% of the respondents. About 1/3 of the respondents indicated that they suffer from chronic diseases (34.3%). None of the respondents had been vaccinated against COVID-19, and 56.5% declared their willingness to be vaccinated. Of those declaring that they did not want to be vaccinated, the main reason was that they did not like the type of vaccine offered to healthcare professionals (18.5%) (Table 3).

Among people suffering from COVID-19, 15.7% declared that their health status did not return to the pre-disease state, and the most frequently declared complications were: neurological and psychiatric (7.4%), general (6.5%), and cardiovascular (5.6%).

3.3. Lifestyle

Further questions were related to lifestyle changes during the pandemic. As many as 77.8% of respondents declared that they felt stress related to the pandemic: 79.8% of women and 68.4% of men. The main cause of anxiety was concern for their own health and that of their family: this was declared by 81.5% of respondents. Interestingly, as many as 42.6% of school employees indicated that the cause of stress was concern about the level of the teaching of their students. Hygiene habits changed during the pandemic. As many as 86.1% of school employees declared wearing the mask in public places, and 73.1% indicated that they disinfect their hands more often (Table 4).

Only one person indicated that they quit smoking during the pandemic, regular smoking was declared by 7.4% of respondents, and 3.7% assessed that they smoked occasionally (Table 4).

Overall, 61.1% of respondents indicated that their eating habits did not change during the pandemic, and 18.5% of respondents indicated a negative change (Table 4).

It is disturbing to note that the pandemic significantly affected the physical activity of the respondents: as many as 35.2% of the school employees indicated no activity at all during the pandemic (Table 5).

Table 4. Lifestyle changes during a pandemic ($n = 108$).

Parameter	Total ($n = 108$)	Women ($n = 89$)	Men ($n = 19$)
	n (%)	n (%)	n (%)
Do you feel stress related to the pandemic?			
Yes	84 (77.8)	71 (79.8)	13 (68.4)
No	24 (22.2)	18 (20.2)	6 (31.6)
What is the stress experienced during a pandemic related to? (multiple choice question)			
Concern for own and family's health	88 (81.5)	73 (82.0)	15 (78.9)
Limited social life	42 (38.9)	36 (40.4)	6 (31.6)
Care for job stability and earnings	31 (28.7)	26 (29.2)	5 (26.3)
Online learning and limited access to computer hardware	18 (16.7)	17 (19.1)	1 (5.3)
Concern for the level of the teaching of their students	46 (42.6)	40 (44.9)	6 (31.6)
Other	2 (1.9)	2 (2.2)	0 (0.0)
How have your hygiene habits changed during the pandemic? (multiple choice question)			
They have not changed	15 (13.9)	13 (14.6)	2 (10.5)
I wash my hands more often	72 (66.7)	59 (66.3)	13 (68.4)
I disinfect my hands more often	79 (73.1)	66 (74.2)	13 (68.4)
I wear the mask in public places	93 (86.1)	76 (85.4)	15 (78.9)
Other	3 (2.8)	2 (2.2)	0 (0.0)
Do you smoke cigarettes?			
Yes, regularly	8 (7.4)	3 (3.4)	5 (26.3)
Yes, occasionally	4 (3.7)	3 (3.4)	1 (5.3)
No	95 (88.0)	83 (93.2)	12 (63.1)
I have smoked, but quit during the pandemic	1 (0.9)	0 (0.0)	1 (5.3)
How do you evaluate the change in eating habits during the pandemic?			
Positive change	22 (20.4)	20 (22.5)	2 (10.5)
Negative change	20 (18.5)	13 (14.6)	6 (31.6)
No change	66 (61.1)	55 (62.9)	11 (57.9)

Table 5. Physical activity before and during a pandemic ($n = 108$).

Physical Activity	Before the Pandemic *	During a Pandemic
	n (%)	n (%)
Lack of physical activity	23 (21.3)	38 (35.2)
1–2 times a week, minimum 30 min	43 (39.9)	42 (38.9)
3–5 times a week, minimum 30 min	25 (23.1)	17 (15.7)
More than 5 times a week, minimum 30 min	17 (15.7)	11 (10.2)

* $p < 0.05$ —statistically significant differences between groups.

3.4. Consumption of Products

We also assessed how eating habits changed during the pandemic. We found significant increases in the consumption of water (21.3%); fruits, vegetables, and salads (20.4%); groats, rice, and cereals (19.4%); tea (19.4%); fish and fish products (16.7%); honey and bee products (15.7%); nuts (11.1%); and eggs (10.2%) (Table 6).

Table 6. Changes in product consumption during a pandemic ($n = 108$).

Product	Increase in Consumption	Decrease in Consumption
	n (%)	n (%)
Fruits, vegetables, salads	22 (20.4) **	3 (2.8)
Honey and bee products	17 (15.7) ***	1 (0.9)
Nuts	12 (11.1) *	3 (2.8)
Milk and dairy products	12 (11.1)	5 (4.6)
Meat and meat products	12 (11.1)	17 (15.7)
Fish and processed fish	18 (16.7) **	3 (2.8)
Eggs	11 (10.2) *	2 (1.9)
Bread	7 (6.5)	14 (13.0)
Groats, rice, cereals	21 (19.4) **	7 (6.5)
Flour preparations (pies, pancakes, rolls, cookies)	18 (16.7)	13 (12.0)
Sweets	21 (19.4)	15 (13.9)
Ready-made dishes for quick preparation at home	7 (6.5)	25 (23.1) **
Coffee	16 (14.8)	13 (12.0)
Tea	21 (19.4) **	6 (5.6)
Juices	10 (9.3)	12 (11.1)
Water	23 (21.3) ***	4 (3.7)
Alcohol	3 (2.8)	12 (11.1) *

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ —statistically significant differences between groups.

A favorable observation was that the consumption of ready-made dishes for quick preparation at home (23.1%) and alcohol (11.1%) decreased (Table 6).

In addition, we recorded an increase in the number of meals consumed during the day: before the pandemic, only 0.9% said they consumed more than five meals; and during the pandemic, this percentage was as much as 13.0%. The pandemic had a significant impact on the time spent in front of the computer. Before the pandemic, as many as 54.7% indicated that they spent less than 2 h in front of the computer a day. During the pandemic, the highest percentage (31.5%) reported spending 6 to 8 h in front of the computer. The highest percentage of school employees reported sleeping from 7 to 9 h (70.4% vs. 65.7%) (Table 7).

Table 7. Changes in eating during a pandemic ($n = 108$).

Parameter	Before the Pandemic	During a Pandemic
	n (%)	n (%)
Number of meals during the day		
1–2 meals	11 (10.2)	10 (9.2)
3–5 meals	96 (88.9)	84 (77.8)
over 5 meals	1 (0.9)	14 (13.0)
Time spent in front of the computer		
less than 2 h a day	59 (54.7) ***	18 (16.7)
2–3 h a day	31 (28.7)	15 (13.9)
4–5 h a day	10 (9.2)	21 (19.4)
6–8 h a day	3 (2.8)	34 (31.5)
more than 8 h a day	5 (4.6)	20 (18.5)
Hours of sleep per day		
6 h or less	31 (28.7)	33 (30.6)
7–9 h	76 (70.4)	71 (65.7)
10 or more hours	1 (0.9)	4 (3.7)

*** $p < 0.01$ —statistically significant differences between women and men.

3.5. Vaccination

Assessment of the IgG antibody level in the group of school employees before vaccination ($n = 108$) showed that 53.7% of the subjects had low levels of antibodies. In the group of people who were vaccinated in the second stage, an antibody level below 18 U/mL was recorded in 51%. The first dose resulted in a high level of protection (antibody levels above 22 U/mL were recorded in 89.8% of school employees), and after the second dose, this was recorded in 100% of respondents (Table 8). Similar data (percentage of people) were obtained when calculating the percentage of people who responded positively after vaccination (Table 8). This table presents the percentage of people depending on the value of the IP parameter.

Table 8. Percentage of people by the IgG and by the rate of positive reaction after vaccination.

Parameter	Before Vaccination ($n = 108$)	Before Vaccination ($n = 49$)	After First Vaccination ($n = 49$)	After Second Vaccination ($n = 49$)
	n (%)	n (%)	n (%)	n (%)
IG				
Under 18 U/mL	58 (53.7)	25 (51.0)	5 (10.2)	0 (0.0)
18–22 U/mL	8 (7.4)	4 (8.2)	0 (0.0)	0 (0.0)
Above 22 U/mL	42 (38.9)	20 (40.8)	44 (89.8)	49 (100.0)
Index of Positivity				
Under 0.9	58 (53.7)	25 (51.0)	5 (10.2)	0 (0.0)
0.9–1.1	8 (7.4)	4 (8.2)	0 (0.0)	0 (0.0)
Above 1.1	42 (38.9)	20 (40.8)	44 (89.8)	49 (100.0)

An interesting observation was the significant increase in the FRAP level after the first vaccination and after the second vaccination, compared to the baseline level (1484.0 and 1581.0 vs. 1428) (Table 9).

Table 9. FRAP level in the study group ($n = 49$).

Parameter	Av. \pm SD	Med. (Q1–Q3)	p
FRAP—before vaccination (A)	1453.3 \pm 292.2	1428.0 (1271.0–1599.0)	$p_{A/B} < 0.0001$
FRAP—after 1 dose (B)	1539.7 \pm 285.0	1484.0 (1346.0–1721.0)	$p_{A/C} < 0.0001$
FRAP—after 2 doses (C)	1613.1 \pm 294.4	1581.0 (1423.0–1787.0)	$p_{B/C} < 0.0001$

Av.—average, FRAP—ferric reducing antioxidant power, Med.—median, Q1–Q3—quartile 1–quartile 3, SD—standard deviation.

We also saw a significant increase in antibody levels in the first dose and second dose groups compared to pre-vaccination antibody levels (400.0 and 270 vs. 17.8 U/mL) (Table S3).

The preventive vaccinations carried out protected almost the entire studied population of school employees from COVID-19 infection: only one person declared infection after the first dose of the vaccine. The main symptoms in this person were high fever and cough. The people we tested did not have the level of antibodies determined by another laboratory. After vaccination, the main symptoms were: forearm pain (81.6%); muscle aches and fatigue (59.1); and shivering and feeling cold (49.0%). Symptoms in most of the respondents started between 7 and 12 h (59.1% of respondents), and usually disappeared after 24 h (59.2% of respondents) (Table S4).

In the further stage of data analysis, we divided the school staff who had been vaccinated into two groups: one group did not reach the maximum level of antibodies that could be demonstrated by the test, and the other group reached the maximum level of IgG antibodies. We found statistically significant differences only in the case of the antibody level after the first dose of vaccination (140.0 vs. 400, $p < 0.001$), and between the IP index

before vaccination (0.635 vs. 1.920, $p < 0.001$) and after the first vaccination (2.670 vs. 4.380, $p < 0.001$). These two groups were very similar in terms of anthropometric parameters (Table S5).

We noticed no differences in the change in body weight between the two groups, as well as in the type of work performed, and thus, a different possibility of contact with potential pathogens (Table S6).

Our research confirmed the link between lower antibody levels and no previous COVID-19 disease. Other factors, such as a positive COVID-19 test among household members, and chronic diseases, had no effect. A disturbing observation is the fact that 24% of respondents with the maximum level of antibodies reported that after infection, they had not yet recovered to their pre-disease state of health (Table S7).

Both groups declared the occurrence of pandemic-related stress to a similar degree, and the main reason was concern for their own health and that of their family (83.3% and 80.0%, respectively). A positive change in eating habits was declared by 25.0% and 20.0% of the respondents, respectively (Table S8).

The frequency of undertaking physical activity was also not related to the achieved antibody level: no statistical significance was shown (Table S9).

People who achieved the antibody level of 400 U/mL indicated, *inter alia*, an increased consumption of fruit, vegetables, and salads, and honey and bee products; however, we noticed that the change in consumption of the analyzed product categories was not related to the body's response to vaccination (Table S10).

We noted a difference between the time spent in front of the computer before the pandemic between the two study groups: 62.4% spent less than 2 h a day in front of a computer in the pre-pandemic period, and in the second study group, this was 44.0% (Table S11).

In the group that did not have peak antibody levels after the second dose, one person developed COVID-19 between the first and second doses. Among post-vaccination symptoms, hand pain was more frequent in the group that reached the maximum antibody level (88.0% vs. 75.0%), but these differences were not statistically significant (Table S12).

By analyzing the correlations between the studied parameters, we saw a significant, very high positive correlation between the level of FRAP before vaccination and after the first dose ($R = 0.92$, $p < 0.0001$), before vaccination and after the second dose ($R = 0.93$, $p < 0.0001$), and after the first dose and the second dose ($R = 0.97$, $p < 0.0001$). An interesting, but difficult to explain, observation is the positive correlation between the FRAP level and anthropometric parameters, such as body weight, height, and BMI (Table 10).

Table 10. Correlations between the selected parameters in the case of people who achieved the maximum level of IgG antibodies, and those with a lower level.

Group	Parameter 1	Parameter 2	R, p
IgG level below 400 (n = 24)	FRAP before vaccination	FRAP after first dose	0.92, 0.0001
	FRAP before vaccination	FRAP after second dose	0.93, 0.0001
	FRAP after first dose	FRAP after second dose	0.97, 0.0001
	IgG before vaccination	IgG after first dose	0.56, 0.0042
	IgG before vaccination	IgG after second dose	0.55, 0.0053
	FRAP after second dose	Growth	0.41, 0.0482
IgG level 400 (n = 25)	FRAP before vaccination	FRAP after first dose	0.76, 0.0001
	FRAP before vaccination	FRAP after second dose	0.78, 0.0001
	FRAP before vaccination	BMI	0.61, 0.0013
	FRAP after first dose	FRAP after second dose	0.98, 0.0001
	FRAP after first dose	BMI	0.68, 0.0002
	FRAP after second dose	BMI	0.70, 0.0001

4. Discussion

Teachers and other school employees are a very important social group exposed to daily contact with a large group of young people. For this reason, they should take special care of their health, as well as apply preventive measures.

Our study had two main goals: to assess teachers' and other school employees changes in eating and health habits during the COVID-19 pandemic, and to assess their response to immunization against this viral infection.

During the pandemic, changes in the daily functioning of societies around the world were observed [33]. Phenomena such as limiting social meetings, uncertain financial situations, work, and distance learning contributed to an increase in the levels of stress, which, in turn, translated into changes in the generally understood lifestyle [34]. In this study, 39% of respondents saw an increase in body weight from 3 to 5 kg during the pandemic, whereas 47.2% of respondents did not notice a change. Weight gain was also observed in other studies [19,35–38]. Only 8% of the subjects lost weight in the range of 6–10 kg. Both weight loss and weight gain could be related to stress. Studies have noticed a negative impact of isolation on well-being and eating behaviors [39,40]. It is well known that stress can affect caloric intake in two ways: some people skip meals, whereas some eat more because of stress [41]. In a study by Zachary et al. (2020), as many as 52% of respondents increased food consumption in response to stress [42]. A study by Pellegrini et al. (2020) also noted a correlation between weight gain and increased levels of anxiety/depression [43]. The increased level of anxiety during the pandemic also increased the risk of eating disorders [38,44]. Increased food consumption in some studies concerned overweight, obese, and elderly people, which may have already resulted from previous bad eating habits [19,45,46]. In this study, the average BMI value indicated overweight, which, according to the cited studies, may increase the risk of maintaining bad eating habits during the pandemic. Weight gain during COVID-19 may also be associated with decreased activity. In this study, the majority of respondents declared a decrease in physical activity as a result of closed gyms, swimming pools, or other sports-related places. The increase in body weight could therefore be related to the disproportionate consumption of calories to the amount of energy expended. Other studies have found no change, a decrease, as well as an increase in physical activity during the COVID-19 pandemic [37,47–49].

Regarding the change in food consumption, there was a statistically significant increase in the consumption of water (23%); fruit and vegetables (22%); tea (21%); groats, rice, and cereals (21%); fish and processed fish (18%); honey and bee products (17%); nuts (12%); and

eggs (11%). Conversely, there was a decrease in ready-to-eat products (25%) and alcohol (12%) compared to what was consumed before the pandemic. Increased consumption of vegetables and fruits may result from greater care for the supply of essential vitamins for fear of viral infection. Studies by Silva et al. (2021) and Salman et al. (2021) also noted an increase in the consumption of vegetables and fruits during the pandemic [49,50]. It is worth noting that the elderly during the pandemic were characterized by a greater decrease in healthy food consumption than the younger generations [51]. Increased consumption of carbohydrate sources has also been noted in other studies [19,20,45]. The increase in the consumption of fish, honey, bee products, and nuts, as in the case of vegetables, could be due to the desire to ensure immunity. Fish, especially sea fish, are a source of valuable anti-inflammatory omega-3 fatty acids. Their positive effect on the immune functions of the body has been shown [52]. Silva et al. (2021) also observed an increase in fish consumption [49]. Honey, bee products, and nuts are also a source of valuable antioxidants, which could have been important when selecting these products [30,53]. An interesting finding is the increased consumption of eggs, which was also noticed by other researchers [54]. The decline in the consumption of ready-made products seems to be a natural phenomenon, because due to remote work and being locked at home, preparing meals was not as difficult as before the pandemic. Additionally, it may have been associated with a desire to save money in uncertain times. In a study by Molina-Montes et al. (2021), 57.8% of participants reduced the consumption of fast-food dishes, and 52% cooked more often [55]. Other studies also report a decrease in the consumption of ready meals [45,49,54]. A positive change that was observed is the increase in water consumption (23%). Changes in the consumption of tea and alcohol were also significant. Contrary to the study by Błaszczuk-Bebenek et al. (2020) [54], a decrease in alcohol consumption by 12% was observed. Similar conclusions were also drawn by Silva et al. (2021) and Ammar et al. (2020) [49,56]. This may be due to the limitation of social gatherings, as noted by Rehm et al. (2020) [57]. The 2021 review shows the overall increase in alcohol consumption during the COVID-19 pandemic [58]. On the other hand, no changes in coffee consumption were observed, whereas a statistically significant increase in tea consumption was noted, which is in line with the review by Castellana et al. (2021). As the authors emphasize, tea is associated with relaxation, concentration, and being at home [59]. The increase in the consumption of sweets was not statistically significant. Other researchers obtained different results [45,49,54]. An increase in the consumption of sweets was also noted in the review by Gonzalez-Monroy et al. (2021) [58]. Their increased consumption could be associated with an increase in stress accompanying the pandemic [60]. The number of meals per day in this study did not change. In other studies, an increase in food consumption was shown [45,54,61], as well as in snacking [54].

It is well known that a proper diet is necessary to maintain proper immunity. An adequate supply of antioxidant vitamins (vitamin C, β -carotene, vitamin E, and vitamin D), as well as minerals (zinc, selenium, copper, and manganese), polyphenols, and omega-3 fatty acids is particularly important. They regulate the immune system, and thus, reduce the risk of infection. A healthy diet rich in fiber also has a positive effect on the intestinal microbiota, which is extremely important in terms of immunity [62].

Sleep also plays an important role in the context of the body's immunity. Sleep is important to rapidly combat antigens by cytotoxic NK cells, which peak in the waking period, and to repair damaged body tissues [63]. Sleep time did not change during the pandemic among study participants. However, sleep quality is important and, as noted by Wrigth et al. (2021), may worsen during a pandemic due to the increased level of anxiety [64]. An inadequate amount and quality of sleep also affects eating behavior [65]. The time spent in front of the computer has also increased. Before the pandemic, it was less than 2 h in 59% of the study participants, whereas during the pandemic, only 18% of the participants declared this response. This is mainly due to school activities and remote work. This translates into a decrease in physical activity, which was noticed by other researchers [36].

An interesting observation was that we found a significant increase in FRAP in people who took the first and second doses of the vaccine. The mechanism of this reaction should be clarified in future research. For example, a decrease in FRAP levels has been observed in patients with active Crohn's disease (0.01 mmol/g of protein) compared to FRAP in patients with inactive disease (0.02 mmol/g of protein) and controls (0.02 mmol/g of protein) [66].

Contreras et al. (2020) conducted a study of the response of animals (cattle) after vaccination against ticks. They measured antioxidant response biomarker parameters, such as: cupric reducing antioxidant capacity (CUPRAC), ferric reducing ability of the plasma (FRAP), trolox equivalent antioxidant capacity (TEAC), total thiol concentrations, and uric acid. The oxidation status was also studied: ferrous oxidation-xylenol orange (FOX), total oxidant status (TOS), advanced oxidation protein products (AOPP), and hydrogen peroxide (H_2O_2). A significant decrease in oxidizing markers was observed, with the exception of thiol. The authors concluded that those vaccines that are capable of inducing lower oxidative stress allow the production of higher levels of antibodies [67]. In our study, we observed a high level of FRAP in the group after vaccination 2, which was related to the fact that all people had a positive response and a correspondingly high level of antibodies.

Moreover, we found a positive correlation between FRAP and anthropometric parameters, such as height, weight, and BMI. An increase in FRAP in morbidly obese patients was observed by Choromańska et al. (2020) [68]. This can be explained by an increase in uric acid, which is an endogenous antioxidant. This acid accounts for up to 80% of the total antioxidant potential. In a physiological concentration, it is the most important plasma antioxidant, whereas in higher concentrations, it can generate free radicals (it has pro-inflammatory and pro-oxidative properties). Elevated levels of antioxidant parameters, such as FRAP, may indicate greater ability to remove free radicals, and effective protection against oxidative stress.

In this study, it was found that higher levels of antibodies after vaccination were correlated with higher levels of FRAP, i.e., the body's ability to reduce iron (III) ions. This is an extremely important observation. However, it is difficult to draw definitive conclusions whether it was a diet rich in antioxidants and, at the same time, increasing the antioxidant status of the body that could have influenced a better response to vaccination. There is a lack of research on this topic. A study on piglets showed promising results, in which it was found that the supplementation of antioxidants with hydrated sodium-calcium aluminosilicates (HASC) increased the level of antibodies after vaccination against the porcine reproductive and respiratory syndrome virus. The supplement consisted of vitamin A and E at a dose of 20,000 IU and 200 IU/kg feed, respectively, as well as selenized yeast at a dose of 0.3 mg/kg, and grape seed extract at a dose of 100 mg/kg feed [69].

Changes in eating habits, as well as physical activity, during the COVID-19 pandemic have been noticed by researchers in various parts of the world. It seems that these changes could have been particularly intensified in the initial phase of the pandemic, due to increased stress related to insufficient adaptation to completely new social and economic conditions. It is important to raise public awareness of healthy eating and its impact on the body's immunity. It appears that the antioxidant status of plasma may have a potential impact on increased immune response to vaccination. This is a new issue, and therefore requires careful research.

The positive correlation observed by us between the FRAP level and anthropometric parameters may indicate the need for further research on the nutritional status of the organism, and the relationship with the parameters of oxidative stress.

There are some limitations to this study. Although it was carried out on the largest group available, further studies should be carried out with a larger number of volunteers. Anthropometric measurements were entered independently by the respondents (due to the pandemic). In further studies, measurements should be performed by a specialist, e.g., a dietitian. Another limitation is the disproportion between the number of women and men. Future research should be gender-balanced. The disproportion in our study reflects the actual gender distribution of school workers.

5. Conclusions

The period of the COVID-19 pandemic contributed to a decrease in physical activity among primary school teachers and other school employees, as well as to an increase in the amount of time spent in front of the computer. As this is a conscious and educated group, a compensation for this could be a change in eating habits, including increased consumption of vegetables, fruits, salads, honey and bee products, nuts, fish and processed fish, eggs, groats, rice, cereals, tea, and water; and reduced consumption of ready-made dishes for quick preparation at home, and alcohol. The protective vaccination against COVID-19 contributed to a significant increase in the level of IgG antibodies. There has also been a significant increase in FRAP, but this issue requires further investigation on the link and determination of whether a higher level is a cause or effect.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/foods11030408/s1>. Table S1: A survey that was carried out in the first stage of the study. Table S2: A survey that was carried out in the second stage of the study. Table S3: Level of IgG in the study group ($n = 49$). Table S4: Respondents' reaction to vaccination with the first dose ($n = 49$). Table S5: Comparison of selected parameters between people who achieved the maximum level of IgG antibodies (400 U/mL) and the lower level ($n = 49$). Table S6: Comparison of selected aspects of lifestyle between people who achieved the maximum level of IgG antibodies and the lower level ($n = 49$). Table S7: Comparison of selected aspects of symptoms, quarantine and opinions on vaccinations during the COVID-19 pandemic between people who achieved the maximum level of IgG antibodies (400 U/mL) and the lower level ($n = 49$). Table S8: Comparison of selected aspects of well-being and habits during the COVID-19 pandemic between people who achieved the maximum level of IgG antibodies (400 U/mL) and the lower level ($n = 49$). Table S9: Comparison of physical activity during the COVID-19 pandemic between people who achieved the maximum level of IgG antibodies (400 U/mL) and the lower level ($n = 49$). Table S10: Comparison of changing eating habits between people who achieved the maximum level of IgG antibodies (400 U/mL) and the lower level ($n = 49$). Table S11: Comparison of changing eating habits between people who achieved the maximum level of IgG antibodies (400 U/mL) and the lower level ($n = 49$). Table S12: Post-vaccination information with 1 dose - comparison between people who achieved the maximum level of IgG antibodies and the lower level ($n = 49$).

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Publikacja H5

Article

Intake of Antioxidant Vitamins and Minerals in Relation to Body Composition, Skin Hydration and Lubrication in Young Women

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Abstract: The aim of this study was to estimate the consumption of selected dietary components with antioxidant properties, undertake body composition analysis, assess skin hydration and lubrication, and establish the relationships between the above parameters. The study was carried out on 172 young women. The consumption of ingredients (vitamins A, C, D and E, and Cu, Mn, Zn) was assessed using the Diet 6.0 program, body composition was assessed using electrical bioimpedance and skin hydration and lubrication were assessed using the corneometric and sebumetric methods, respectively. About one-third of students showed insufficient consumption of vitamin C, vitamin E and zinc, while about 99% showed insufficient vitamin D levels. The highest degree of hydration was observed in the areas of the eyelids, neckline and chin. The greatest amount of sebum was found in the area of the nose and forehead. Low positive correlations between hydration or lubrication and Cu, vitamin A and vitamin E were observed. In conclusion, to properly moisturize and lubricate the skin, young women should eat products that are rich in ingredients with antioxidant properties, in particular fat-soluble vitamins A and E, but also copper.

Keywords: vitamin A; vitamin C; vitamin D; vitamin E; copper; manganese; zinc; body composition; skin; antioxidants

1. Introduction

The skin is a barrier that separates the human body from the external environment. It takes part in many processes, including metabolic and immune processes, and has a thermoregulatory and protective function against pathogenic microorganisms. This organ can fulfill its functions only when it is moistened and the hydro-lipid layer is properly composed. Skin condition is influenced by a number of factors, including genetic and environmental factors (diet, smoking and weather conditions, among others) [1,2]. Many of these factors can disrupt the oxidative/reductive status.

Both extracellular and intracellular oxidative stress, which are initiated by reactive oxygen species, can result in skin pigmentation disorders, as well as premature aging. Overexposure to ultraviolet (UV) radiation can accelerate this process [3].

A properly balanced diet should provide ingredients from all categories, namely, basic nutrients, vitamins and minerals, in accordance with the standards established by experts [4,5]. An important role in the pathogenesis of many diseases, including skin diseases, is played by the deficiency of ingredients with antioxidant properties [6–9].

Disturbed oxidative balance can cause premature skin aging but also lead to the development of diseases, such as acne, rosacea, skin cancer, psoriasis, vitiligo, scleroderma, contact dermatitis, lichen planus and chronic venous ulcers [10].

Vitamin A is involved, among others, in the protection against the effects of reactive oxygen species, in the proper functioning of the immune system, in cell division and differentiation and in maintaining their proper structure. It should be emphasized that this vitamin contributes to the normal maintenance condition of the epidermis, *inter alia*, by regulating the process of exchange and exfoliation of the outer layers of cells. The external application of retinol may contribute to the reduction of wrinkles. Moreover, retinol helps to increase the accumulation of hyaluronic acid in the epidermis, which plays an important role in moisturizing the skin. Retinoids inhibit transepidermal water loss (TEWL), improve the protective function of the epidermis, prevent collagen degradation and can be a chemopreventive agent [11–14].

Vitamin C is water soluble, participates in collagen biosynthesis and helps in antioxidant protection in the case of photodamage caused by UV radiation. In addition, it prevents scurvy and degenerative diseases [15,16]. Moreover, it affects the absorption of calcium and iron consumed with meals or pharmaceutical preparations. Additionally, vitamin C helps to seal the capillaries [4,17].

Vitamin D is primarily involved in calcium phosphate metabolism, for example, bone metabolism. Studies have shown the presence of vitamin D receptors in extra-skeletal organs, including the brain, heart and intestines. The pleiotropic effect of vitamin D was tested in numerous studies and the results suggest a relationship between its low concentration in blood serum and an increased risk of cancer, immune-related diseases, immune disorders, civilization diseases, psychiatric disorders and neurodegenerative diseases [18–20]. Furthermore, research confirms its diverse functions and role in the etiology of various dermatological diseases, such as acne, atopic dermatitis and melanoma [21,22].

Vitamin E has an anti-aging effect. This vitamin has the ability to neutralize free radicals in a hydrophobic environment. Literature data indicate that it may protect against the risk of developing, for example, atherosclerotic lesions, coronary artery disease [23,24], cataracts and cancers [25]. It is used in the form of both oral supplementation and topical therapy due to its photoprotective and skin-barrier-stabilizing properties [26].

Copper (Cu) is a part of superoxide dismutase, the main enzyme involved in the breakdown of free radicals. It takes part in the formation of bonds in collagen and elastin, as well as in the synthesis of melanin, which is the dye of hair and skin [27].

Manganese (Mn) activates enzymes that are involved in the synthesis of proteins, fatty acids and nucleic acids, and also participates in the metabolism of thyroid hormones. It is included in, among others, superoxide dismutase (SOD), protecting the body against free radicals. It is necessary for the proper formation of connective tissue and the appearance of the skin [28].

Zinc (Zn) has regulatory, structural and catalytic functions, and it is a component of over 300 enzymes, for example, Cu/Zn-SOD. This microelement participates in the metabolism of essential nutrients, such as proteins, fats and carbohydrates, as well as in energy metabolism and the functioning of many hormones. The skin has the third-highest zinc content in the human body. Zinc deficiency is associated with diseases such as pellagra, delayed wound healing and alopecia. In addition, it regulates the secretion of sebum, has anti-inflammatory and anti-blackhead properties and is used in the treatment of acne [4,29].

The above dietary ingredients with antioxidant properties have a positive effect on the processes taking place in the skin. The aim of the study was to assess the consumption of ingredients with antioxidant properties (vitamins A, C, D and E, and Cu, Mn and Zn), body composition and hydration and lubrication of the skin. Moreover, this study aimed to determine whether the dietary intake of antioxidants or body composition correlated with skin condition. As far as we know, it is the first publication of this type that combines the above parameters.

2. Materials and Methods

2.1. Study Group

The study was conducted among young women ($n = 172$) who were students of laboratory medicine, pharmacy, cosmetology and dietetics at the Medical University of Białystok, Poland. All participants were residents of cities with more than 300,000 inhabitants. The women were between 18 and 25 years old, their heights ranged from 155 to 182 cm and their body weights ranged from only 39 to 100 kg (Table 1). All study participants declared that they did not smoke cigarettes.

Table 1. Characteristic of study group ($n = 172$).

Parameters	Av. \pm SD	Min–Max	Med.	Q1–Q3
Age (years)	20 \pm 1	18–25	20.00	21–21
Height (cm)	167 \pm 6	155–182	168.00	163–171
Weight (kg)	62 \pm 10	39–100	60.60	54–68
BMI (kg/m ²)	21.9 \pm 3.2	15.3–34.7	21.35	19.8–24.0

Av.—average, Max—maximum, Med.—median, Min—minimum, Q1—lower quartile, Q3—upper quartile, SD—standard deviation.

All the students agreed to perform the analyses. The study was approved by the Bioethics Committee of the Medical University of Białystok (R-I-002/8/2015 and R-I-002/39/2015).

2.2. Estimating the Consumption of Antioxidant Ingredients

Three 24 h nutritional interviews were conducted with each student using the current record method, namely, based on ‘Album of photographs of food products and dishes’ [30]; the interviews included one free day and 2 working days.

Based on the nutritional interviews, the intake of vitamins and minerals with antioxidant properties was calculated using the Diet 6.0 computer program. This program was developed by the National Food and Nutrition Institute in 2018 [4,31,32]. It includes over 2100 products and dishes, and approximately 1300 dietary supplements.

The obtained results were compared with the currently applicable standards at the levels of: estimated average requirement (EAR) and adequate intake (AI). Factors such as age, gender and physiological condition were taken into account. The results were compared to the norm at the EAR level in the case of: vitamin A (500 μ g retinol equivalent (RE)), vitamin C (60 mg), Cu (0.7 mg) and Zn (6.8 mg), and at the level of AI in the case of vitamin D (15 μ g of cholecalciferol), vitamin E (8 mg of α -tocopherol equivalent) and Mn (1.8 mg) [4]. Moreover, the percentage of people with insufficient and sufficient consumption was calculated.

EFSA recommendation requirements differ from national or regional standards: vitamin A (average requirement (AR): 570 μ g RE), vitamin C (AR: 90 mg), vitamin E (AI: 13 mg of α -tocopherol equivalent), Cu (AI: 1.3 mg), Mn (AI: 3.0 mg) and Zn (AR: 6.2 mg). In the case of vitamin D, the recommendation is the same (AI: 15 μ g of cholecalciferol) [5].

2.3. Body Composition Analysis

Body composition was assessed using the InBody 720 (Biospace, Eonju-ro, Korea) device, which is based on the bioelectrical impedance analysis (BIA) method. It is medical equipment with high accuracy. The principle of the method is based on measuring the body’s resistance. The human body is divided into 5 cylindrical parts, which are fed with currents of different voltages. The device uses 8-point electrodes to improve the accuracy of the measurement. This method is safe, non-invasive and reliable.

During the study, the following parameters were measured: indicative of physical activity (fitness score), circuits (abdomen circumference, arm muscle circumference, chest circumference, hip circumference), indicating the content of bone minerals (bone mineral content), concerning the basal metabolism (basal metabolic rate), the content of muscle

and fat tissue (body fat mass, fat-free mass, mineral mass, obesity degree, percent body fat, protein mass, skeletal lean mass, skeletal muscle mass, visceral fat area) and water content in different areas (extracellular fluid/total body fluid: ECF/TBF, extracellular water/total body water: ECW/TBW, extracellular water mass, intracellular water mass, total body water mass).

2.4. Skin Preparation and Measurement Conditions

Before starting the study, the students rested for 10–20 min in order to stabilize blood circulation and reduce the impact of physical activity on skin hydration and lubrication. The areas of skin (cheeks, chin, eyelids, forearm, forehead, neckline, nose) on which the measurements were made were previously cleaned with a make-up remover and then with water at home on the day of the analysis.

The measurements were performed in a room with a temperature of about 20 °C and air humidity ranging from 40 to 60%.

2.5. Measurement of Skin Hydration

Skin moisture was measured with a Corneometer CM 825 (Courage + Khazaka Electronic, Köln, Germany). This is a capacitive method. The measurement is based on the difference between the dielectric constant of a substance (usually below 7) and water (81). Depending on the changing water content, the measuring capacitor shows changes in capacitance. There are 2 tracks in the probe head. An electric field is created between them. One path has an electron deficiency (positive sign) and the other path has an electron excess (negative sign). When measuring skin moisture, the scattering field penetrates the first layer (10–20 µm of the stratum corneum) of the skin and the capacitance is determined on this basis. Three measurements were taken in each area and the result is presented as an average.

A score below 30 units (u.) pointed to very dry skin, from 30 to 40 meant that the skin was dry and above 40 meant that the skin was determined as sufficiently moisturized [33].

2.6. Measurement of Skin Lubrication

The skin lubrication was measured with the Sebumeter SM 815 (Courage + Khazaka Electronic, Köln, Germany). It is a direct, photometric method that is based on the measurement of sebum secreted on the skin. A measuring tape with an area of 64 mm² and placed in the cassette was applied to the skin for 30 s. The measuring head of the cassette was placed in the aperture of the device and the photocell measured the transparency of the tape. In the studied areas, 3 measurements were made and the final result is the average of these readings.

The obtained result ranged from 0 to 350 µg/cm². Depending on the examined area, the following criteria were adopted for the degree of skin lubrication: dry skin (reading <100 µg/cm² on the forehead and in the T zone, <70 µg/cm² on the cheeks), normal (from 100 to 200 µg/cm² on the forehead and in the T zone, from 70 to 180 µg/cm² on the cheeks), oily (over 220 µg/cm² on the forehead and in the T zone, over 180 µg/cm² on the cheeks) [33].

2.7. Statistical Analysis

The obtained numerical data were analyzed using Microsoft Office Excel 2019 and Statistica 13.3 Software (StatSoft, Tibco, Palo-Alto, CA, USA). The normality of the data distribution was assessed using the Kolmogorov–Smirnov, Lilliefors and Shapiro–Wilk tests. Descriptive statistics parameters were also calculated: mean, standard deviation, median, minimum, maximum and lower and upper quartiles.

The Kruskal–Wallis ANOVA test was used for the statistical analysis. Spearman's rank-order correlation coefficients were also determined. The level considered as statistically significant was $p < 0.05$.

3. Results

It was shown that students were mainly characterized by insufficient intake of vitamin C (39.5% of the students) and zinc (32.0%). Sufficient intake of vitamin D was found in only 1.2% and sufficient vitamin E in 26.7% (Table 2).

Table 2. Percentage of young women with sufficient and insufficient consumption of the tested antioxidant ingredients.

Component	Type of Norm	Norm (per Day)	Consumption Av. \pm SD	Students with Insufficient Consumption (%)	Students with Sufficient Consumption (%)
Vitamin A (μ g RE)	EAR	500	825.0 \pm 688.1	27.3	-
Vitamin C (mg)	EAR	60	82.6 \pm 54.1	39.5	-
Vitamin D (μ g cholecalciferol)	AI	15	2.7 \pm 2.9	-	1.2
Vitamin E (mg α -tocopherol equivalent)	AI	8	6.9 \pm 4.1	-	26.7
Cu (mg)	EAR	0.7	1.0 \pm 0.4	20.9	-
Mn (mg)	AI	1.8	3.9 \pm 1.9	-	91.9
Zn (mg)	EAR	6.8	8.1 \pm 2.3	32.0	-

AI—adequate intake, Av.—average, EAR—estimated average requirement, RE—retinol equivalent, SD—standard deviation.

Table 3 presents the descriptive statistics for the female body composition analyses. The average fitness score was 72.77 ± 5.08 points, which indicates that this group was moderately active. Moreover, it was shown that the intracellular water mass was on average 20.09 ± 2.33 L and extracellular water mass was 12.32 ± 1.44 L. It was shown that the mean value of the total body water mass parameter was 32.40 ± 3.75 kg. Importantly, it was also noted that the ECF/TBF averaged 0.333 ± 0.004 and the ECW/TBW averaged 0.380 ± 0.005 .

Table 3. Descriptive characteristics of the body composition parameters estimated by electrical bioimpedance method.

Parameter	Av. \pm SD	Min–Max	Med.	Q1–Q3
Total body water mass (kg)	32.40 \pm 3.75	23.40–44.90	32.25	29.55–35.05
Extracellular water mass (L)	12.32 \pm 1.44	9.00–17.40	12.20	11.20–13.20
Intracellular water mass (L)	20.09 \pm 2.33	14.40–27.50	20.05	18.30–21.75
ECW/TBW	0.380 \pm 0.005	0.363–0.392	0.380	0.377–0.384
ECF/TBF	0.333 \pm 0.004	0.317–0.345	0.334	0.330–0.337
Fat free mass (kg)	44.27 \pm 5.15	31.80–61.20	44.05	40.30–47.90
Skeletal lean mass (kg)	41.62 \pm 4.82	30.00–57.50	41.40	37.90–45.10
Skeletal muscle mass (kg)	24.20 \pm 3.04	16.77–33.90	24.13	21.90–26.35
Protein mass (kg)	8.68 \pm 1.01	6.20–11.90	8.70	7.90–9.40
Body fat mass (kg)	17.34 \pm 6.65	5.40–44.50	16.45	12.15–21.15
Percent body fat (%)	27.39 \pm 6.47	13.90–45.50	27.56	22.40–31.40
Mineral mass (kg)	3.18 \pm 0.39	2.19–4.39	3.14	2.89–3.45
Bone mineral content (kg)	2.65 \pm 0.34	1.84–3.66	2.62	2.42–2.86
Basal metabolic rate (kcal)	1326.19 \pm 111.18	1057.71–1240.70	1321.35	1240.70–1405.05
Visceral fat area (cm ²)	58.89 \pm 23.13	10.20–142.20	56.08	42.95–69.38
Abdomen circumference (cm)	78.89 \pm 8.83	58.30–111.30	77.45	72.65–84.30
Hip circumference (cm)	93.62 \pm 5.61	80.10–114.7	92.95	89.65–96.95
Chest circumference (cm)	88.29 \pm 5.93	73.90–110.00	87.55	84.10–92.10
Arm muscle circumference (cm)	22.57 \pm 1.58	18.85–27.77	22.57	21.43–23.60
Fitness score (points)	72.77 \pm 5.08	56.00–84.00	73.00	70.00–76.00

Av.—average, ECF—extracellular fluid, ECW—extracellular water, Max—maximum, Min—minimum, Q1—lower quartile, Q3—upper quartile, SD—standard deviation, TBF—total body fluid, TBW—total body water.

As part of this study, the hydration of selected areas of the body and face was assessed. It was shown (Figure 1) that individual areas differed significantly in terms of their water content in the epidermis ($*** p < 0.001$). The eyelids (67.02 ± 11.35 u.), neckline (62.14 ± 10.41 u.) and chin (59.66 ± 10.90 u.) had the highest mean water contents in the epidermis, and the nose (26.55 ± 16.21 u.) had the lowest water content.

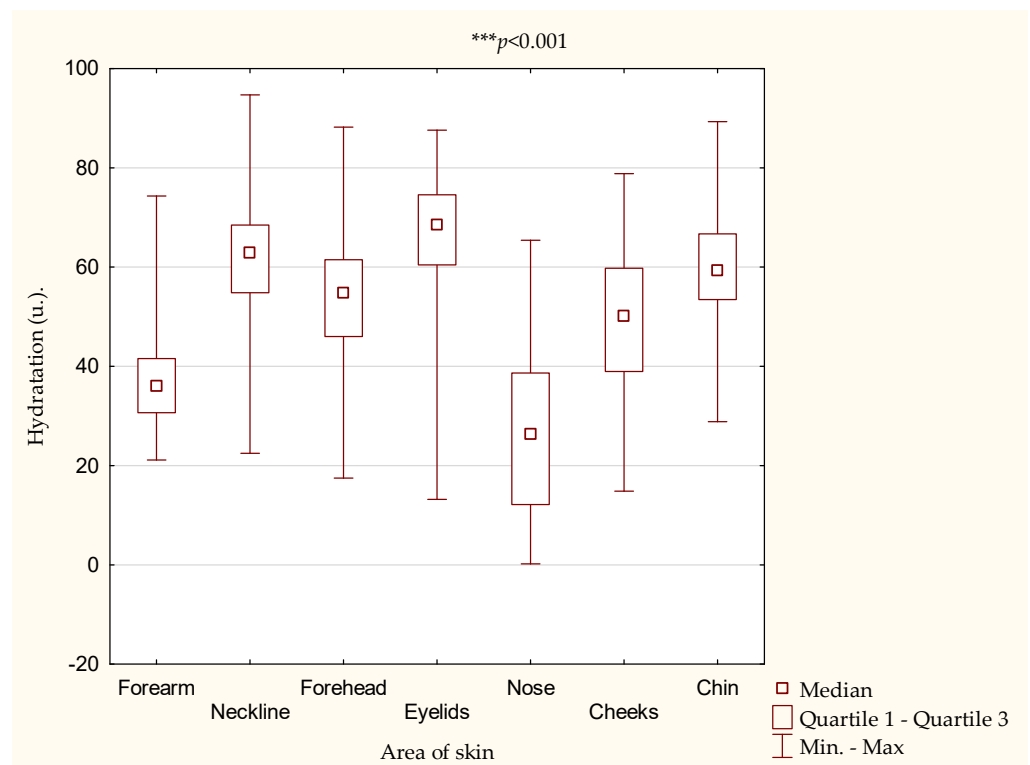


Figure 1. Differences in hydration of the body and face skin. Av.—average, SD—standard deviation.

The assessment of the lubrication of the face skin showed that the most sebum was present on the skin of the nose ($105.13 \pm 73.53 \mu\text{g}/\text{cm}^2$) and forehead ($103.62 \pm 65.69 \mu\text{g}/\text{cm}^2$). The degree of lubrication significantly depended on the area of the skin (Figure 2).

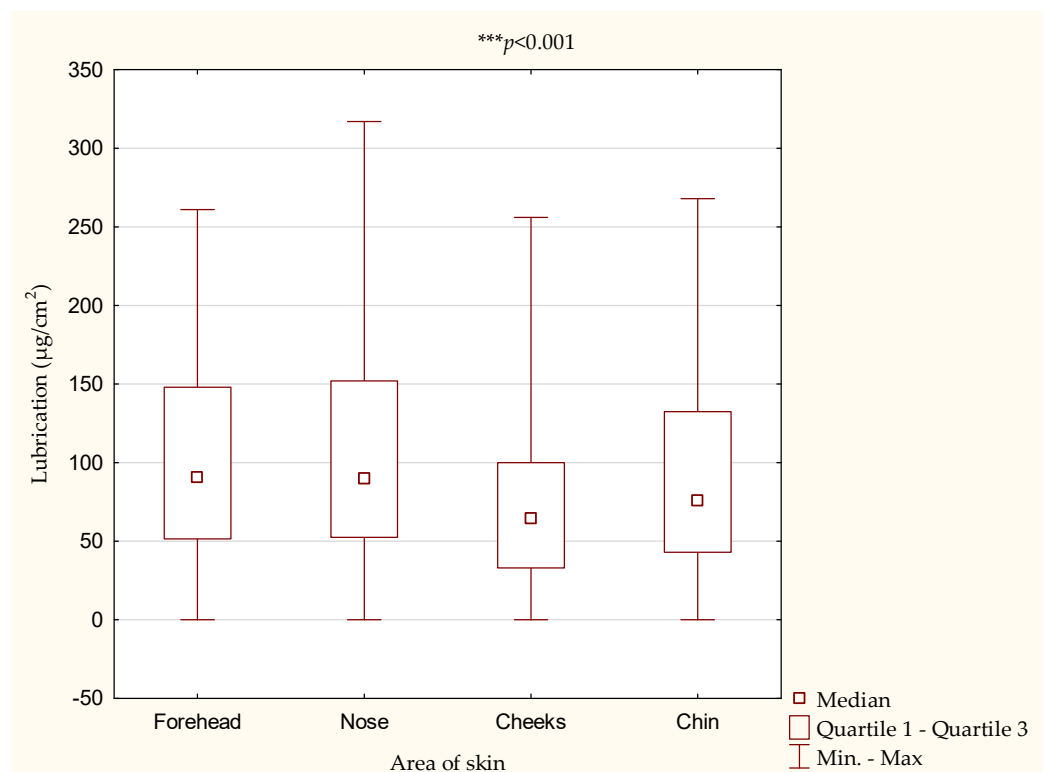


Figure 2. Differences in lubrication of the body and face skin. Av.—average, SD—standard deviation.

In the next step, an assessment of what percentages of the female students were characterized by each of the three levels of skin hydration—very dry, dry and sufficiently moisturized—in individual areas took place. It was shown that the highest percentage of female students had sufficient hydration of the eyelids (98.2%), neckline (97.6%) and chin (96.0%). The nose was the area that had very dry skin for the largest group of female students (59.3%) (Table 4).

When assessing the lubrication, the results were classified into three categories: dry, normal and oily skin. It was shown that the highest percentage of female students was characterized by dry skin on the chin (64.5%) (Table 5).

Table 4. Percentage of young women with each degree of hydration of the examined skin areas.

Area	Women with Skin Type (%)		
	Very Dry	Dry	Sufficiently Moisturized
Cheeks	7.6	18.0	74.4
Chin	0.6	3.4	96.0
Eyelids	1.2	0.6	98.2
Forearm	23.3	45.3	31.4
Forehead	3.5	8.1	88.4
Neckline	1.2	1.2	97.6
Nose	59.3	19.2	21.5

Table 5. Percentage of young women with each degree of lubrication of the examined skin areas.

Area	Women with Skin Type (%)		
	Dry	Normal	Oily
Cheeks	53.5	39.5	7.0
Chin	64.5	33.2	2.3
Forehead	55.8	37.8	6.4
Nose	57.6	33.7	8.7

It was shown that in the case of skin hydration, the vast majority of female students had average skin hydration, defined as sufficient ($n = 166$), and only $n = 6$ female students had dry skin.

Different results were obtained in the case of skin lubrication. It was shown that $n = 102$ female students had dry skin, $n = 69$ women had normal skin, and only $n = 1$ had oily skin. Therefore, for the analysis, the purpose of which was to show differences in the consumption of ingredients with antioxidant properties, two groups were selected: people with dry ($n = 102$) and normal skin ($n = 69$). Interestingly, in the case of women with normal skin, higher average consumption of all tested ingredients was noted, but in no case were these differences statistically significant (Figure 3).

The assessment of the correlation between the consumption of the analyzed vitamins and minerals with antioxidant properties showed significant correlations between almost all diet components (Table S1 in the Supplementary Material). The highest relationships were noted between the consumption of Cu and Zn ($r = 0.74$), Cu and Mn ($r = 0.66$) and Mn and Zn ($r = 0.62$).

Relationships between the hydration and lubrication of examined skin areas were also searched for (Table S2 in the Supplementary Material). There were no correlations between total hydration and total lubrication, only correlations between single areas. For example, there was a moderate positive correlation between the hydration of the forearm and neckline.

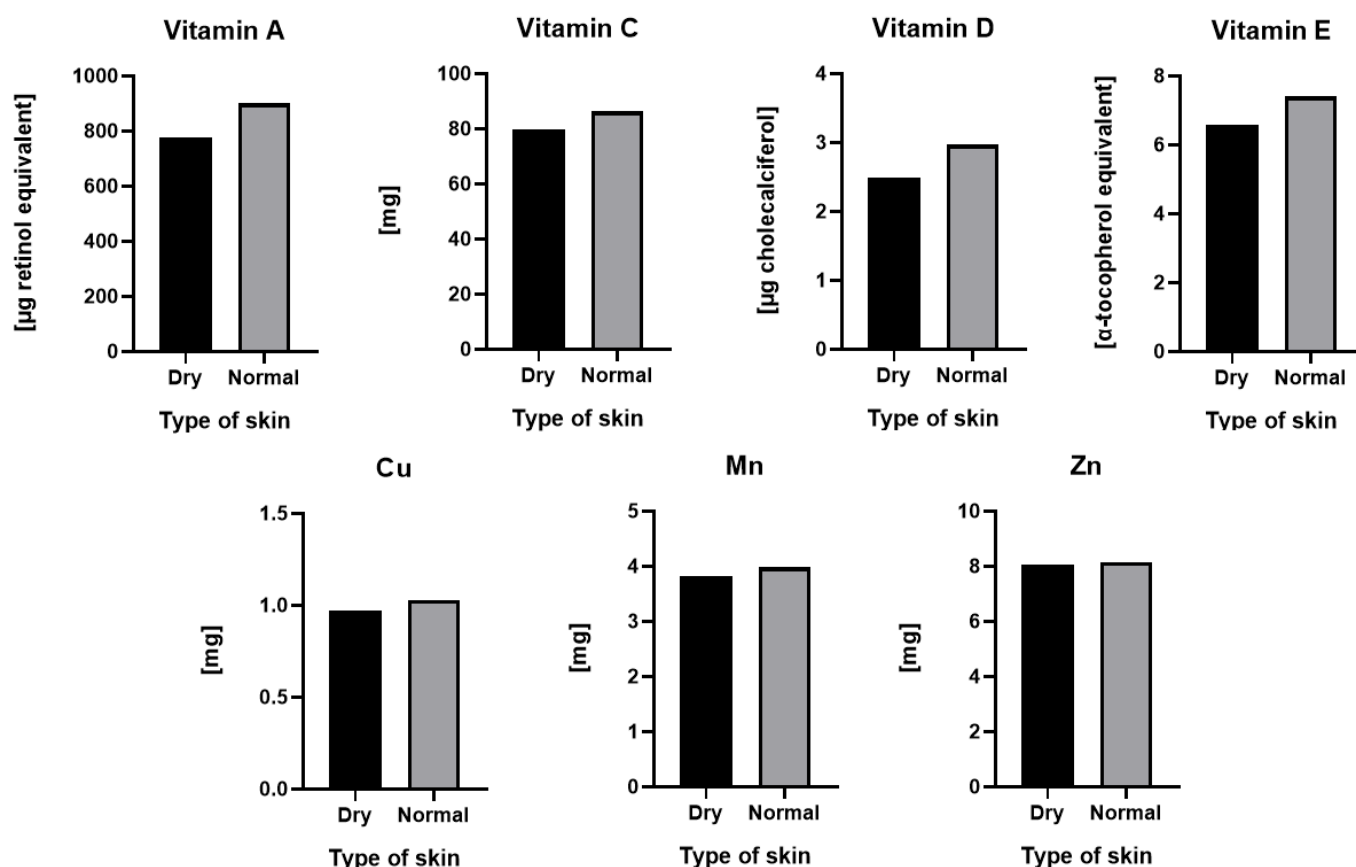


Figure 3. Consumption of antioxidant ingredients by young women with dry ($n = 102$) and normal ($n = 69$) skin: vitamin A, vitamin C, vitamin D, vitamin E, Cu, Mn and Zn.

Additionally, the relationship between skin hydration and lubrication and the results of body composition analysis were assessed in the group of female students ($n = 172$). There was a negative correlation between age and cheek skin hydration ($r = -0.19$), as well as between body moisturizing (forearm and neckline skin) and ECW/TBW ($r = -0.15$). Positive correlations occurred in the case of the lubrication of skin and ECF/TBF ($r = 0.15$) (Table 6).

Table 6. Correlations between skin hydration, lubrication and body composition ($p < 0.05$).

Factor 1	Factor 2	<i>r</i>
Body moisturizing	ECW/TBW	−0.15
Moisturizing the cheeks	Age	−0.19
Total lubrication	ECF/TBF	0.15

ECF—extracellular fluid, ECW—extracellular water, TBF—total body fluid, TBW—total body water.

The relationship between skin hydration and lubrication and the consumption of selected ingredients with antioxidant properties was most evident in the case of vitamins A and E, as well as Cu. There was a low positive correlation between total skin hydration and vitamin A consumption ($r = 0.17$) and a low positive correlation between skin lubrication and vitamin E consumption ($r = 0.15$) and Cu consumption ($r = 0.17$) (Table 7).

Table 7. Correlations between skin hydration, lubrication and intake of antioxidant components ($p < 0.05$).

Factor 1	Factor 2	<i>r</i>
Vitamin A	Hydration of the neckline	0.16
Vitamin A	Hydration of the forearm	0.16
Vitamin A	Hydration of the face	0.18
Vitamin A	Total hydration	0.17
Vitamin E	Hydration of the neckline	0.17
Vitamin E	Hydration of the face	0.16
Vitamin E	Total lubrication	0.15
Cu	Hydration of the face	0.16
Cu	Total lubrication	0.17

4. Discussion

This study focused on the search for relationships between skin hydration and lubrication, body composition and the consumption of ingredients with antioxidant properties. Lubrication and hydration of the skin can be directly related to nutritional status and body composition analysis results are indirectly related to eating habits. Literature data show a strong relationship between nutrition and skin condition, but little research has focused on the relationships investigated in this publication.

Properly moisturized skin creates a barrier that protects organs and tissues against mechanical, chemical and biological factors [34]. There are four basic types of skin in the literature: dry, normal, oily and combination [2].

Dry skin is a type of skin with a high degree of epidermal dehydration. It is characterized by an abnormal process of keratinization and exfoliation of epidermal cells and impaired lipid production, which contributes to the formation of dryness [35]. Our research showed that 102 female students had dry skin.

Normal skin is rarely found in young women. This type of skin is characterized by smoothness and firmness, as well as the absence of skin defects [36]. According to the results of our study, 69 young women had normal skin.

Oily skin is characterized by excessive activity of the holocrine sebaceous glands. This type usually occurs in young people and during adulthood. Excess sebum on the surface of the skin and keratinization disorders can lead, among others, to the development of acne [37]. This type of skin was identified in only one person.

Combination skin is the most popular type of skin and is a combination of dry and oily skin. The dry U zone is distinguished (around the eyes and temples) and the oily T-zone (forehead, nose, cheeks, chin). This type is characterized by disturbed hydration on the epidermis surface [37].

Each type of skin requires carefully selected care, as well as a diet that is aimed at reducing defects and possibly supporting the treatment of skin diseases.

We showed that the areas of the body and face of the young women differed significantly in terms of skin hydration, and the areas that were best moisturized for the majority of them were the eyelids (98.2% of women), neckline (97.6% of women), chin (95.9% of women) and forehead (88.4%) (Table 4).

In the case of skin lubrication, we also noted statistical differences between the examined skin areas. Normal skin was recorded on the cheeks in 39.5% of the examined women and on the forehead in 37.8% of the women (Table 5).

As part of this study, the impact of the consumption of selected dietary components with antioxidant properties was assessed: vitamins A, C, D and E, as well as Cu, Mn and Zn.

Vitamin A is one of the basic antioxidant vitamins. As shown in the present study, the average daily consumption was 825 ± 688 μ g retinol equivalent (RE) and 27.3% of young women were characterized by a low dietary intake of this vitamin (Table 2). In the case of deficiency, the diet can be enriched with products such as: meat, butter, dairy products and

eggs, as well as vegetables and fruits (carrots, red peppers, dark green leafy vegetables, melons and mangoes) [38]. The EFSA report summarizes data gathered from national reports about the intake of nutrients in European countries. According to the EFSA average, vitamin A intake ranged between 816 and 1498 $\mu\text{g RE/day}$ in adults [5]. Gacek [39] conducted a study among 120 women aged 19–25 who regularly engaged in physical activity. The supply of vitamin A was at the level of $706.8 \pm 111.2 \mu\text{g/day}$ (117.8% of the norm). The research carried out in our study showed that both women with dry skin and women with normal skin had a higher intake of vitamin A (777.03 vs. 901.66 $\mu\text{g/day}$, respectively). A study conducted in a group of 1004 young women aged 20–34 showed that the average consumption of vitamin A was as much as 1017 μg , which was a value that was higher by 240 $\mu\text{g/day}$ than among the women in our study that were characterized by dry skin [40]. Research conducted by Gogojewicz et al. [41] on women aged 20–40 showed that the average consumption of vitamin A in the group of women practicing fitness ($n = 20$) was $488.1 \pm 336.1 \mu\text{g}$ (the norm coverage was at the 69.7% level), while in the control group: $719.3 \pm 963.8 \mu\text{g}$ (102.7% of the norm).

The analysis of consumption showed that the average dietary intake of vitamin C reached $82.6 \pm 54.1 \text{ mg/day}$ and almost 40% of the women consumed too little vitamin C. Our results are similar with data obtained in other European countries, where the average vitamin C intakes range from 65 to 138 mg/day in women [5]. A study conducted on young women [39] showed that the average vitamin C intake was $78.5 \pm 15.2 \text{ mg/day}$ (130.9% of the norm). This level was similar to the level of consumption by students from our research that had dry skin. Other authors estimated for the group of young women ($n = 1004$) that the average consumption of vitamin C was 79.1 mg/day , which was a value similar to the consumption of vitamin C by young women with dry skin in this present study [40]. In the group of young women practicing fitness ($n = 20$), as well as in the control group ($n = 20$), a low consumption of vitamin C was found at the levels of $32.9 \pm 19.5 \text{ mg/day}$ and $36.1 \pm 21.6 \text{ mg/day}$, respectively, which accounted for 44.0% and 48.0% of the implementation of the standards [41]. These values were much lower than those shown in our study. It is commonly believed that citrus fruits and in their juices (lemon, oranges, grapefruits, bergamot) are products that are rich in vitamin C; however, much larger amounts can be found in blackcurrant, chokeberry, tomatoes, green and red peppers, strawberries, kiwifruit and green leafy vegetables, such as broccoli [42,43].

In the case of vitamin E, the average daily dietary intake ($6.9 \pm 4.1 \text{ mg}$) was lower than AI (8 mg) and only 26.7% of students ingested enough of it. Due to this fact, the intake of the following products should be increased: vegetable oils (sunflower, safflower) and nuts (almonds, hazelnuts) [25,44]. According to the EFSA report [5], in other countries, the average α -tocopherol intake was higher and ranged between 7.8 and 12.5 mg/day in women. The average consumption of vitamin E in local studies was at the level of $7.7 \pm 1.4 \text{ mg/day}$ (98.6% of the norm), which was a value higher than the consumption in both groups studied by us [39]. The other study on young women from Poland ($n = 1004$) showed that the average daily vitamin E intake was higher (9.4 mg) [40] than in our study. The consumption of vitamin E in a group of 20 young women from Poland doing fitness exercises was shown to be at the level of $4.1 \pm 1.3 \text{ mg/day}$, while in the control group, it was at the level of $5.4 \pm 2.0 \text{ mg/day}$, which were 51.2 and 67.8% of the standard, respectively [41]. The average consumption was lower than that of the female group in our study.

Only 1.2% of the surveyed women ingested a sufficient amount of vitamin D with the diet. It would be recommended to enrich the diet with products that are good sources of vitamin D, such as fish (tilapia, salmon and herring) [45]. Research confirms that fish consumption (300–1000 g per week) increases the concentration of vitamin D in serum [46]. However, the main factor responsible for the vitamin D content in the body is cutaneous synthesis [47]. Therefore, vitamin D intake is rarely assessed. For example, studies conducted in a group of 161 women showed that the average vitamin D intake was

at the level of 2.45–2.92 µg/day, meaning that the percentage of compliance with the norm was 49% [48]. Our research showed a much larger anomaly.

In our research, we found that the consumption of Cu, Mn and Zn was 1.0 ± 0.4 , 3.9 ± 1.9 , 8.1 ± 2.3 mg/day, respectively. Insufficient intake was the most severe for Zn consumption (32%). Our results are similar to the EFSA report [5], where the average Cu intakes ranged between 1.15 and 2.07 mg/day, the mean Mn intakes of adults generally ranged around 3 mg/day (from 2 to 6 mg/day) and the average Zn intake ranged from 8.0 to 14.0 mg/day in adults. Omeljaniuk et al. [49] showed that the average consumption of Cu at the level of 0.87 ± 0.3 mg/day, Mn: 3.48 ± 1.6 mg/day, and Zn: 7.58 ± 2.3 mg/day. The percentages of women with insufficient consumption were 18%, 18% and 45% for Cu, Mn and Zn, respectively. Another study conducted among 161 students showed the consumption of Cu at the level of 0.86 ± 0.31 mg/day (which was 95.2% of the norm), the consumption of Mn at the level of 3.61 ± 1.56 mg/day (200.5% of the norm) and the consumption of Zn was 7.25 ± 2.40 mg/day (90.5% of the norm) [48]. Our previous research showed an interesting relationship: physically active students were characterized by higher consumptions of, among others, Cu, Mn and Zn. In the group of students who did not exercise but who were physically active, the values were the following: in the case of Cu— 1.09 ± 0.7 mg/day vs. 1.22 ± 0.6 mg/day), in the case of Mn— 4.68 ± 2.3 mg/day vs. 5.09 ± 2.0 mg/day, while in the case of Zn— 9.66 ± 4.9 mg/day vs. 12.47 ± 5.9 mg/day [50]. However, our research showed insufficient intake of Cu in 20.9% of the group. Too low Zn intake was reported in almost one-third of the young women. The most beneficial result for the consumption of ingredients with antioxidant properties was obtained for Mn. It was shown that 91.9% of the respondents ingested a sufficient amount of this ingredient with their diet.

Body composition analysis is a common, non-invasive method for assessing fat and lean body mass. Body composition analyzers differ, among other things, in the degree of advancement, the number of parameters tested and their accuracy. Haq et al. [51] assessed, inter alia, body composition in a group of students of medical universities in China. The study was conducted on 695 students, including 471 females. In the group of young women, the BMI was at the level of 21.6 ± 3.7 kg/m², while the fat percentage was $28.5 \pm 7.9\%$. The group of young women we studied ($n = 172$) had a similar BMI (21.9 ± 3.2 kg/m²) (Table 1) and percentage of body fat ($27.39 \pm 6.47\%$) (Table 3).

Contemporary studies assessed the relationship between body composition and the emergence of civilization diseases, as well as skin diseases. However, there are still few scientific reports on the influence of body composition on the hydration and lubrication of the skin. In our work, we tried to assess these dependencies.

The control of body weight and parameters determining body composition is important because excessive fat gain adversely affects the functioning of organs and well-being. Often it is also the basis for the development of diseases, such as type 2 diabetes, insulin resistance and hypertension, which consequently also affect the appearance of the skin.

We have shown that better skin hydration is associated with a lower index of edema. Proper hydration results in a sufficient amount of extracellular water, while when its supply is disturbed, excess fluid may accumulate in the intercellular space, causing swelling. The opposite tendency occurs in the case of the assessment of lubrication of skin, which is indicated by a positive correlation.

The corneometric and sebumetric methods that we used are the reference methods. In the literature, they are mainly used to: compare other methods to them [52,53], the impact of supplementation (e.g., collagen [54], evaluation of the effectiveness of new formulations and uses of ingredients [55–57] and the external use of preparations [58–61]). To our knowledge, this is the first time that they have been used to comprehensively assess the impact of a diet on skin hydration and lubrication. Moreover, there are publications describing their repeatability and accuracy [62] and characterizing them in detail [63].

There are many methods for assessing the condition of skin, e.g., methods for assessing exposure to environmental pollution and oxidative stress, pigmentation, topography,

radiance and color, elasticity, density, firmness and the structure of the microbiome [64]. These methods can be used to work with patients, as well as for research purposes. For example, there are reports suggesting that higher levels of the antioxidant substance lycopene in the skin correlate with lower roughness of skin [65].

In order for the epidermis to perform its function, it requires the presence of a hydro-lipidic film on the surface and an appropriate degree of hydration to condition itself in response to external and internal factors. The effect of using dietary ingredients with antioxidant properties was assessed by Heinrich et al. [66]. The duration of the study was 12 weeks. The first group of volunteers ($n = 13$) received lycopene (3.0 mg), lutein (3.0 mg), beta-carotene (4.8 mg), alpha-tocopherol (10.0 mg) and selenium (75.0 mg); the second group ($n = 13$) received lycopene (6.0 mg), beta-carotene (4.8 mg), alpha-tocopherol (10.0 mg) and selenium (75.0 mg); the third group ($n = 13$) received a placebo. The above supplementations influenced the density and thickness of the epidermis. In the case of the density, the authors noted statistically significant changes of 6.57% (in the first group) and 7.01% (in the second group). The thickness analysis showed statistically significant changes of 15.99% in the first group and 14.09% in the second group.

Literature data show that both free radicals generated under the influence of UV radiation and those generated as a result of endogenous production can lead to photoaging of the skin [67], and in extreme cases, even to skin cancer [68]. Substances with antioxidant properties can be used externally or ingested. Local application of this type of substances allows them to be delivered directly to the skin surface in full without losses. Although the goal of the pharmaceutical and cosmetology industries is to increase the penetration of substances into the deeper layers of the skin, it is not high enough. On the other hand, oral ingestion of ingredients with antioxidant properties allows for reaching the deeper layers of the skin [69].

To sum up, our research shows that skin hydration was significantly influenced by the consumption of vitamins A and E, and skin lubrication was influenced by the consumption of Cu. Vitamin A and its derivatives contribute to the proper exfoliation of the stratum corneum, which improves its protective function and reduces transepidermal water loss [70]. Vitamin E, on the other hand, is one of the most effective antioxidants. By penetrating deep into the lipid barrier of skin cells, vitamin E seals and strengthens the cell membrane, which causes water retention [26]. The mechanism of the influence of Cu on skin lubrication has not, to our knowledge, been characterized in the literature. It is emphasized that Cu stimulates the proliferation of skin fibroblasts. It is a cofactor of superoxide dismutase and also prevents lipid peroxidation and oxidative damage to cell membranes [71].

The advantage of this study is the assessment of the actual intake of antioxidant ingredients with the standard diet of the study participants. Other studies, such as that by Dumoulin et al. [72], relied on participants taking the formulation developed by the authors. Our research allowed for assessing the effects of consumed antioxidants on skin hydration and lubrication; this can be the basis for rational supplementation that is tailored to the needs of patients. When the diet is rich in antioxidants, the use of additional dietary supplements seems unnecessary.

It should also be emphasized that the choice of products, diet balancing and eating habits of patients are influenced by a number of factors, such as social status; economic, cultural and adaptive factors; and interest in a healthy lifestyle [73–76]. Nutritional factors are one of the selected aspects because the proper hydration of the skin can also be influenced by genetic factors and not just a properly balanced diet.

Our study has several limitations. The analyses were carried out in a group of young women; it is worth also conducting them in a group of young men, but they show less willingness to participate in this type of research. The three-day dietary interview method of current recording is a very common method for assessing the consumption of selected ingredients, but it is subject to error due to the self-estimation of portion sizes by the participants. Research on the influence of factors on the level of skin hydration and

lubrication may in the future also include a survey on skin care and the amount of water consumed by respondents, as well as assess the consumption of polyphenolic compounds.

Another aspect that needs to be investigated is the assessment of skin hydration and lubrication in other areas, such as the feet. This is especially important in diseases such as diabetes. Patients with this disease should take special care of their skin. The skin of the feet is characterized by the thickest layer of stratum corneum. It is dry and has a tendency toward crack formation. This favors the penetration of microorganisms, which results in infection [77].

Future research should focus on evaluating other factors that affect proper skin hydration and lubrication, as well as the correlation between the degree of skin hydration and lubrication and the risk of skin diseases.

5. Conclusions

Over one-third of students showed insufficient consumption of products rich in vitamins C, D and E, as well as zinc. The highest degree of hydration was observed in the areas of the eyelids, neckline and chin. However, the greatest amount of sebum was found in the areas of the nose and forehead. With increasing age, the level of cheek hydration decreased. A low positive correlation between hydration or lubrication and Cu, vitamin A and vitamin E was observed. In conclusion, to properly moisturize and lubricate the skin, young women should eat products that are rich in ingredients with antioxidant properties, in particular, fat-soluble vitamins A and E, but also copper.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/antiox10071110/s1>, Table S1: Correlations between the consumption of ingredients with antioxidant activity ($p < 0.001$), Table S2: Correlations between skin hydration and lubrication ($p < 0.05$).

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







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Publikacja H6

Article

Consumption of Food Supplements during the Three COVID-19 Waves in Poland—Focus on Zinc and Vitamin D

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Abstract: Food supplements (FS) are a concentrated source of vitamins, minerals, or other ingredients with nutritional or other physiological effects. Due to their easy availability, widespread advertising, and sometimes low price, increased consumption of this group of preparations has been observed. Therefore, the aim of the study was to assess the knowledge and intake of FS during the COVID-19 pandemic in Poland, with particular reference to FS containing zinc and vitamin D. It was noted that both of the above ingredients were used significantly more often by people with higher education (59.0%), with a medical background or related working in the medical field (54.5%), and/or exercising at home (60.1%). Preparations containing vitamin D were used by 22.8% of the respondents in the first wave, 37.6% in the second wave, and 32.9% in the third wave. To sum up, we showed the highest consumption of vitamin and mineral supplements, and preparations containing zinc and vitamin D were taken significantly more often by people with higher medical and related education. This indicates a high awareness of health aspects and the need for preventive measures in these groups.

Keywords: food supplements; immunity; COVID-19; zinc; vitamin D; lifestyle; Poland

1. Introduction

According to the definition of the European Food Safety Authority (EFSA), a food supplement (FS) is a foodstuff intended to be a complement to a normal diet and is a concentrated source of nutrients (vitamins, minerals) or other substances with a nutritional or physiological effect. FS could contain specific substances separately or in a complex combination. There are different forms of FS: pills, tablets, capsules, powder sachets, liquid ampoules, dropper bottles, and other. In the European Union, FS are regulated as foods and must be safe to consume. The regulations determine the maximum level for vitamins and minerals and some other substances. However, in Poland only maximum levels of vitamins and minerals are determined. FS could be used to correct nutritional deficiencies or to maintain an adequate intake of nutrients. Their intake is not a substitute for a varied and balanced diet [1]. Prevention and treatment are physiological activities that are not allowed to be attributed to FS. Moreover, the label must not state that they are recommended for the treatment of diseases.

In Poland, the first case of SARS-CoV-2 infection was diagnosed on 4 March 2020. One week later, the World Health Organization declared the COVID-19 pandemic (on 11 March 2020). To date, more than 209 million COVID-19 cases and over 4.4 million deaths have been reported worldwide, while in Poland over the total is 2.8 million cases and 75,000 deaths

(as of 23 August 2021). Three waves of the pandemic can be distinguished in Poland so far. The first was between March and May 2020, the second between September and November 2020, and the third from February to April 2021. Most of the infected patients experienced mild to moderate symptoms such as tiredness, fever, cough, headache, and loss of smell or taste. However, the list of possible symptoms is getting longer due to the research being conducted on this topic. There is a higher risk of severe COVID-19 among the elderly and those suffering from chronic illnesses (e.g., diabetes, cancer, and chronic respiratory diseases) [2].

Adequate diet and nutritional status are key elements in the maintenance of the proper functioning of the immune system. SARS-CoV-2 infection is usually associated with a decreased immune response, leading to pneumonic inflammation. Among the notably important components improving immune functions, vitamins (A, B₆, B₉, B₁₂, C, D, and E), microelements (Fe, Cu, Se, and Zn), and n-3 long-chain polyunsaturated fatty acids (PUFAs) are indicated [3]. It was demonstrated that vitamin D supplementation was related to a lower risk of respiratory infections [4]. In a randomized trial, it was observed that humoral immunity was improved by the supplementation of vitamins A and D among pediatric patients who received influenza vaccination [5]. However, there is still insufficient evidence to provide exact recommendations on vitamin C, D, and E supplementation for the prevention and treatment of COVID-19 [6]. The positive influence of vitamin B₆ on immunity involves activation of innate or adaptive immunity and the influence on the proliferation of immune cells [7]. Zn is important for the development and functioning of neutrophils and natural killer cells [8]. Fe modulates the differentiation and proliferation of T-cells and the production of reactive oxygen species, which take part in removing infectious agents. The influence of PUFAs on viral infections is not well established and requires further research [9]. Sufficient Se intake supports the immune system, while Se deficiency impairs innate and acquired immunity by the negative influence on cellular as well as humoral immunity (i.e., the production of antibodies) [10].

During the pandemic, negative changes in eating habits and lifestyle such as increased consumption of alcohol, sweets, and fast food or reduced physical activity were reported [11,12]. Taking into account the positive aspects, an increased intake of fruits, vegetables, nuts, legumes, and fish was also observed [13]. On the other hand, greater interest in searching for information on improving the immune system by food products or FS was observed [14,15].

In Poland, before the pandemic (in 2017), the worth of the FS market was estimated at 4.4 billion PLN (approximately 113 million USD) and over 70% of Poles used FS. It is estimated that in 2025 the global supplements market will reach 300 billion USD [16].

Currently, several anti-COVID-19 vaccines have been approved for use in humans to protect against the disease. However, vaccination hesitancy or resistance is observed among different populations [17]. Therefore, supporting immunity through adequate nutrition rich in essential nutrients and developing effective therapy against COVID-19 still seem to be crucial.

This study aimed at an assessment of the changes in the FS intake patterns, with a special focus on the supplements influencing immunity during the three waves of the COVID-19 pandemic in Poland.

2. Materials and Methods

2.1. Participants

This study was carried out among 935 Polish residents during three pandemic waves: $n = 236$ people answered questions about the first waves of the pandemic; the second: $n = 364$, and the third: $n = 335$. Each survey was conducted for about one month after the end of the period it covered. The study was conducted from July 2020 to April 2021. Responses of people living abroad ($n = 9$) were rejected. The inclusion criteria were being a resident of Poland, an adult (over 18 years of age), and answering all the questions. Each participant was informed that their participation was completely voluntary, the

questionnaire was anonymous, and they could resign from participation in the study at any time. The researchers did not collect any data that could be used to identify people, including personal data. Each participant was allowed to complete the questionnaire only once. Consent to participate in the study was expressed by writing down the responses and sending them to the researchers.

2.2. Questionnaire

The questionnaire (containing questions and answers) was included as an attachment to the publication. The three questionnaires contained the same questions, but the third contained one additional question concerning the respondents' knowledge about the possibility of preventing viral infections (number 35) (Appendix A).

2.3. Statistical Analysis

Statistical analysis of the results was performed using Statistica software (TIBCO Software Inc., Palo Alto, CA, USA) and calculator for the chi-square test [18]. The dependencies between the qualitative features were assessed using the Chi-square test of independence. The level of significance was $p < 0.05$.

3. Results

Most of the respondents were women (during the first wave: 80.0%, during the second wave: 81.9%, and during the third wave: 79.7%). Our survey was anonymous and voluntary, and we had no option to select a gender group. A larger percentage of women participating in the study may indicate, at the same time, greater interest in aspects of health and social life among people of this gender.

Residents of all 16 voivodeships participated in the study, but the vast majority were inhabitants of Podlaskie and Mazowieckie voivodeships; the remaining inhabitants accounted for less than 5%.

Adults with an average age of 31 ± 11 , 28 ± 9 , and 28 ± 10 years, mainly with higher education (66.9%, 59.3%, and 47.5%, respectively), participated in the survey. Most respondents lived in a large city of over 250,000 inhabitants (37.3%, 40.1%, and 36.1%) or a village (33.9%, 24.9%, and 30.7%). About half of the respondents from each group described their financial situation as rather good (56.8%, 53.3%, and 49.5%), and most households were comprised of 2–4 people. It is noteworthy that, during the first wave of the pandemic, as many as 50.0% of the respondents worked at their usual office or worksite, while during the second and third waves the percentage was lower (38.5% and 36.4%). About three-quarters of the respondents described their level of physical activity as low during each of the three periods (68.2%, 78.8%, and 78.2%) (Table 1).

It was shown that, during the first round of the survey, the highest percentage of respondents described their health as very good (39.8% vs. 27.0% and 21.5%). In the first half of 2020, the respondents significantly more often answered that they did not suffer from COVID-19. It is disturbing that, during the third wave, as many as 40.0% of respondents noticed an increase in their body weight, and only 42.7% undertook physical activity at home (Table 2).

Table 1. Characteristics of the study group.

Variable	First Wave <i>n</i> = 236 % (<i>n</i>)	Second Wave <i>n</i> = 364 % (<i>n</i>)	Third Wave <i>n</i> = 335 % (<i>n</i>)
Gender			
Female	80.0 (189)	81.9 (298)	79.7 (267)
Male	20.0 (47)	18.1 (66)	20.3 (68)
Anthropometric measurements			
Age (years)	31 ± 11	28 ± 9	28 ± 10
Mass (kg)	69 ± 15	69 ± 18	69 ± 15
Weight (m)	1.69 ± 0.08	1.70 ± 0.09	1.70 ± 10
BMI (kg/m ²)	25.06 ± 4.41	23.91 ± 7.45	23.72 ± 4.45
Education			
Primary school	2.1 (5)	1.1 (4)	2.4 (8)
Higher	66.9 (158)	59.3 (216)	47.5 (159)
Secondary	31.0 (73)	39.6 (144)	50.1 (168)
Type of education			
Medical and related	47.5 (112)	56.6 (206)	45.1 (151)
Nonmedical	36.4 (86)	22.3 (81)	25.4 (85)
Not applicable	16.1 (38)	21.1 (77)	29.5 (99)
Place of residence			
City with up to 150,000 inhabitants	21.6 (51)	26.7 (97)	26.3 (88)
City with 150,000–250,000 inhabitants	7.2 (17)	8.3 (30)	6.9 (23)
City with over 250,000 inhabitants	37.3 (88)	40.1 (146)	36.1 (121)
Village	33.9 (80)	24.9 (91)	30.7 (103)
Subjective assessment of the material situation			
Very good	20.8 (49)	25.8 (94)	22.1 (74)
Average	20.8 (49)	20.1 (73)	26.0 (87)
Rather good	56.8 (134)	53.3 (194)	49.5 (166)
Rather bad	1.6 (4)	0.5 (2)	2.1 (7)
Bad	0.0 (0)	0.3 (1)	0.3 (1)
Number of people in the household			
1	4.2 (10)	7.5 (28)	7.8 (26)
2	24.6 (58)	22.8 (83)	20.6 (69)
3	29.2 (69)	25.3 (92)	19.7 (66)
4	24.6 (58)	30.5 (111)	29.9 (100)
5	10.6 (25)	10.2 (37)	13.7 (46)
6	2.5 (9)	2.2 (8)	2.7 (9)
7	2.5 (6)	0.3 (1)	3.6 (12)
8	0.5 (1)	0.8 (3)	1.2 (4)
10	0.0 (0)	0.3 (1)	0.9 (3)
Professional activity			
Unemployed person	7.2 (17)	1.9 (7)	3.6 (12)
Person working in office	50.0 (118)	38.5 (140)	36.4 (122)
Person working remotely	8.5 (20)	8.2 (30)	6.0 (20)
Student	34.3 (81)	51.4 (187)	54.0 (181)
Physical activity			
Inactivity (sedentary)	0.0 (0)	0.0 (0)	0.0 (0)
Low (occasional exercise, 1–3 times a week)	68.2 (161)	78.8 (287)	78.2 (262)
Moderate (1 h of exercise per day)	25.4 (60)	18.9 (68)	17.3 (58)
High (hard physical work and daily workouts)	6.4 (15)	2.3 (9)	4.5 (15)

Table 2. Assessment of health and physical activity during COVID-19.

Variable	First Wave (<i>n</i> = 236)	Second Wave (<i>n</i> = 364)	Third Wave (<i>n</i> = 335)
How would you rate your health at the beginning of the pandemic in Poland?			
Very good	39.8 (94)	27.0 (98)	21.5 (72)
Good	45.3 (107)	58.2 (212)	61.8 (207)
Medium	11.4 (27)	12.9 (47)	14.6 (49)
Poor	3.5 (8)	1.9 (7)	2.1 (7)
Have you had COVID-19?			
Yes	0.0 (0)	11.8 (43)	17.6 (59)
No	86.0 (203) ***	59.9 (218)	48.7 (163)
It is difficult to say unequivocally	14.0 (33)	28.3 (103)	33.7 (113)
Has your body weight changed during the pandemic?			
No	48.3 (114)	49.5 (180)	43.9 (147)
Increased	37.3 (88)	31.9 (116)	40.0 (134)
Decreased	14.4 (34)	18.6 (68)	16.1 (54)
Did you exercise at home during the pandemic?			
Yes	42.4 (100)	48.4 (176)	42.7 (143)
No	57.6 (136)	51.6 (188)	57.3 (192)

Differences between the various pandemic waves: *** $p < 0.001$.

It is satisfactory that almost all respondents correctly answered what a dietary supplement is—that it only supplements nutritional deficiencies (99.2%, 100.0%, and 97.6%)—and know the difference between FS and medications; this answer was indicated by 91.5%, 97.5%, and 95.8%. It is surprising that the most frequently chosen category of FS during all three waves of the pandemic in Poland was vitamin and mineral preparations (40.3%, 60.2%, and 54.3%), and preparations affecting immunity came in second place. It was shown that preparations from this category were consumed by twice as many people during the second and third wave than during the first wave (18.2%, 37.4%, and 34.9%) (Table 3).

During the first wave, a significantly greater percentage of respondents declared not taking food supplements with zinc and vitamin D (63.6% vs. 30.0% and 39.4%), and the most important reason cited for using them was the desire to supplement deficiencies of vitamins and minerals (36.0%, 27.5%, and 54.3%) (Table 3).

The authority of pharmacists' recommendations was noticeable during the second wave—as many as 13.5% of respondents chose these preparations at the recommendation of a pharmacist. An important fact is that the vast majority (over 90%) drink FS and medications with water. As many as 62.3% of respondents declared that they had not noticed an increase in the number of advertisements for FS during the pandemic. The vast majority of respondents used supplements as recommended (58.1%, 70.5%, and 63.6%). More than 85% of respondents (86.9%, 94.2%, and 88.4%) were aware of the side effects, and over 90% of the risk of overdose (91.1%, 96.1%, and 94.0%). It should also be emphasized that over 70% of respondents indicated that FS should be used only in the case of diagnosed deficiencies (70.8%, 77.5%, and 73.4%). A significantly higher percentage (95.9%) indicated an awareness of interactions between FS and medications in the second wave. In the third round of the survey, a question was added regarding awareness of the beneficial effect of the use of preparations containing zinc and vitamin D in the prevention of viral infections—79.7% of respondents indicated that they had heard such reports (Table 3).

Table 3. Assessment of knowledge about food supplements and their consumption during the COVID-19 pandemic.

Variable	First Wave (n = 236)	Second Wave (n = 364)	Third Wave (n = 335)
What is a food supplement?			
A preparation that treats nutritional deficiencies	0.8 (2)	0.0 (0)	2.4 (8)
A preparation that only replenishes nutritional deficiencies	99.2 (234)	100.0 (364)	97.6 (327)
Do you think food supplements differ from medications?			
Yes	91.5 (216)	97.5 (355)	95.8 (321)
No	2.9 (7)	2.2 (8)	2.4 (8)
I do not know	5.6 (13)	0.3 (1)	1.8 (6)
What categories of food supplements did you use during the pandemic?#			
Vitamin–mineral supplements	40.3 (95)	60.2 (219)	54.3 (182)
Probiotics	13.1 (31)	18.1 (66)	15.5 (52)
Prebiotics	3.4 (8)	2.7 (10)	3.3 (11)
Supporting immunity	18.2 (43)	37.4 (136)	34.9 (117)
Supporting weight loss	3.0 (7)	1.9 (7)	3.0 (10)
Improving the condition of the hair, skin, and nails	14.4 (34)	19.5 (71)	22.4 (75)
Supporting the functioning of the urinary tract	2.1 (5)	0.8 (3)	3.3 (11)
Supporting the heart	1.7 (4)	1.4 (5)	4.2 (14)
Supporting memory	2.5 (6)	4.7 (17)	8.4 (28)
Supporting lowering cholesterol levels	1.3 (3)	0.8 (3)	1.2 (4)
Vision support	1.7 (4)	3.0 (11)	3.3 (11)
Supporting the functioning of the joints	5.1 (12)	4.7 (17)	1.8 (6)
Relieving the symptoms of menopause	0.0 (0)	0.0 (0)	0.6 (2)
Supporting the digestive tract	3.8 (9)	3.8 (14)	5.4 (18)
Improving well-being	3.4 (8)	4.4 (16)	4.8 (16)
Facilitating sedation and sleep	6.8 (16)	6.9 (25)	11.3 (38)
Supporting libido	1.3 (3)	0.8 (3)	0.3 (1)
Supporting alcohol metabolism	0.4 (1)	1.4 (5)	0.3 (1)
For athletes	3.8 (9)	2.7 (10)	5.1 (17)
Removing excess water	0.4 (1)	0.0 (0)	0.0 (0)
Other	0.0 (0)	1.6 (6)	0.0 (0)
I did not use dietary supplements	42.8 (101)	19.7 (72)	23.9 (80)
Have you used zinc and vitamin D food supplements since March 2020?			
No	63.6 (150) **	30.0 (109)	39.4 (132)
Only drugs	5.1 (12)	16.5 (60)	13.1 (44)
Yes both	7.2 (17)	14.0 (51)	12.5 (42)
Only zinc	1.3 (3)	1.9 (7)	2.1 (7)
Only vitamin D	22.8 (54)	37.6 (137)	32.9 (110)
Why did you use such food supplements?#			
Not applicable	47.9 (113)	24.7 (90)	26.9 (90)
To improve health	22.2 (52)	21.4 (78)	33.7 (113)
Due to a pharmacist's recommendation	1.7 (4)	13.5 (49)	3.0 (10)
Due to a doctor's recommendation	3.8 (9)	0.0 (0)	0.3 (1)
To supplement deficiencies of vitamins and minerals	36.0 (85)	27.5 (100)	54.3 (182)
To supplement the therapy prescribed by doctor	2.5 (6)	9.9 (36)	10.1 (34)
Due to a friend's recommendation	5.1 (12)	6.0 (22)	3.0 (10)
Because I was encouraged by TV/media/Internet advertising	0.0 (0)	0.3 (1)	1.2 (4)
Other	0.0 (0)	0.0 (0)	2.1 (7)

Table 3. Cont.

Variable	First Wave (n = 236)	Second Wave (n = 364)	Third Wave (n = 335)
What do you usually use to wash down food supplements and medications?#			
Tea	8.1 (19)	11.3 (41)	22.4 (75)
Cola	1.7 (4)	0.5 (2)	1.5 (5)
Not applicable	0.8 (2)	4.7 (17)	6.0 (20)
I do not drink	5.1 (12)	0.5 (2)	1.2 (4)
Juice	3.8 (9)	4.7 (17)	4.8 (16)
Water	93.2 (220)	93.4 (340)	90.1 (302)
Coffee	0.4 (1)	2.5 (9)	4.5 (15)
Milk	0.4 (1)	0.0 (0)	0.9 (3)
Other	0.4 (1)	0.5 (2)	0.6 (2)
Do you think there were more advertisements for food supplements during the pandemic?			
No	5.1 (12)	1.4 (5)	3.6 (12)
Yes	32.6 (77)	42.9 (156)	59.1 (198)
I did not notice a change	62.3 (147) **	55.7 (203)	37.3 (125)
Do you use food supplements in the amount recommended on the package?			
I do not use it	39.9 (80)	19.0 (69)	23.0 (77)
No, I use lower doses	3.8 (9)	4.7 (17)	6.0 (20)
No, I use higher doses	4.2 (10)	5.8 (21)	7.5 (25)
Yes	58.1 (137)	70.5 (257)	63.6 (213)
Do you think food supplements can have side effects?			
No, taking them is absolutely safe	13.1 (31)	5.8 (21)	11.6 (39)
Yes	86.9 (205)	94.2 (343)	88.4 (296)
How do you assess the advisability of using food supplements?			
They should be used only in the event of identified deficiencies	70.8 (167)	77.5 (282)	73.4 (246)
Their use is unnecessary	13.1 (31)	7.7 (28)	9.0 (30)
I have no opinion	16.1 (38)	14.8 (54)	17.6 (59)
Do you think food supplements can be overdosed on?			
No, they're safe	8.9 (21)	3.8 (14)	6.0 (20)
Yes	91.1 (215)	96.1 (350)	94.0 (315)
Do you think that food supplements can interact with medications prescribed by your doctor, and thus affect the effectiveness of therapy?			
No, they're safe	10.2 (24)	4.1 (15)	9.0 (30)
Yes	89.8 (212)	95.9 (349) ***	91.0 (305)
Has the pandemic affected your use of food supplements?			
No	90.7 (214) ***	61.8 (225)	81.8 (274)
Yes, I use fewer	0.4 (1)	1.4 (5)	1.2 (4)
Yes, I use more	8.9 (21)	23.1 (84)	17.0 (57)
I did not use food supplements	0.0 (0)	13.7 (50)	0.0 (0)
Have you heard that preparations containing zinc and vitamin D can support immunity and be helpful in the prevention of viral infections?#			
No	-	-	20.3 (68)
Yes	-	-	79.7 (267)

Differences between the various pandemic waves: ** $p < 0.01$, *** $p < 0.001$, # multiple choice question.

In the following part, the entire study group was divided in terms of the use of FS containing only zinc, only vitamin D, or both. FS with both ingredients were chosen significantly more often by people with higher education (59.0%) and with medical and related education (54.5%) (Table 3). Among the inhabitants of large cities—with a population of over 250,000—the highest percentage of respondents used preparations containing both vitamin D and zinc. Preparations containing only zinc were significantly more often used by people assessing their financial situation as rather good (64.7%) and by students (70.6%). Four-person families used both these components (73.9%) as prophylaxis. The highest percentage of people who suffered from COVID-19 consumed both zinc and vitamin D (no statistical significance); however, the degree of dependence, i.e., whether these ingredients were used before or after infection, was not found (Table 4).

Table 4. Consumption of food supplements with zinc, vitamin D and both ingredients depending on various factors.

Variable	Only Zinc <i>n</i> = 17 % (n)	Only Vitamin D <i>n</i> = 301 % (n)	Both Food Supplements <i>n</i> = 110
Gender			
Female	88.2 (15)	84.4 (254)	77.3 (85)
Male	11.8 (2)	15.6 (47)	22.7 (25)
Education			
Primary school	11.8 (2)	1.0 (3)	0.9 (1)
Secondary	58.8 (10)	41.7 (126)	40.1 (45)
Higher	29.4 (5)	57.3 (172)	59.0 (64) ***
Type of education			
Medical and related	35.3 (6)	50.1 (152)	54.5 (60) *
Nonmedical	29.4 (5)	27.9 (84)	27.3 (30)
Not applicable	35.3 (6)	22.0 (65)	18.2 (20)
Place of residence			
A city with up to 150,000 inhabitants	23.5 (4)	29.6 (89)	20.9 (23)
A city with 150,000–250,000 inhabitants	17.6 (3)	8.3 (25)	3.6 (4)
A city with over 250,000 inhabitants	35.4 (6)	39.2 (118)	47.3 (52)
Village	23.5 (4)	22.9 (69)	28.2 (31)
Subjective assessment of material situation			
Very good	23.5 (4)	23.6 (71)	30.0 (33)
Rather good	64.7 (11) ***	51.8 (156)	18.3 (55)
Average	11.8 (2)	23.6 (71)	15.5 (17)
Rather bad	0.0 (0)	1.0 (3)	4.5 (5)
Bad	0.0 (0)	0.0 (0)	0.0 (0)
Number of people in the household			
1	5.9 (1)	6.6 (20)	1.3 (4)
2	23.5 (4)	26.6 (80)	10.6 (32)
3	11.8 (2)	22.6 (68)	9.3 (28)
4	41.1 (7)	28.6 (86)	73.9 (31)
5	11.8 (2)	10.2 (31)	4.3 (13)
6	0.0 (0)	1.7 (5)	0.3 (1)
7	0.0 (0)	1.7 (5)	0.3 (1)
8	5.9 (1)	1.0 (3)	0.0 (0)
9	0.0 (0)	0.7 (2)	0.0 (0)
10	0.0 (0)	0.3 (1)	0.0 (0)
Professional activity			
Unemployed person	0.0 (0)	3.7 (11)	0.9 (5)
Person working in office	29.4 (5)	39.9 (120)	42.7 (47)
Person working remotely	0.0 (0)	8.3 (25)	8.2 (9)
Student	70.6 (12) *	48.1 (145)	48.2 (49)
Physical activity			
Inactivity (sedentary)	0.0 (0)	0.0 (0)	0.0 (0)
Low (occasional exercise, 1–3 times a week)	70.5 (12)	81.4 (245)	72.7 (80)
Moderate (1 h of training per day)	23.6 (4)	14.6 (44)	20.9 (23)
High (hard physical work and daily workouts)	5.9 (1)	3.9 (12)	6.4 (7)
How would you rate your health at the beginning of the pandemic in Poland?			
Very good	17.6 (3)	27.6 (83)	28.2 (31)
Good	64.7 (11)	57.8 (174)	56.4 (62)
Medium	11.8 (2)	11.9 (36)	11.8 (13)
Poor	5.9 (1)	2.7 (8)	3.6 (4)

Table 4. Cont.

Variable	Only Zinc <i>n</i> = 17 % (n)	Only Vitamin D <i>n</i> = 301 % (n)	Both Food Supplements <i>n</i> = 110
Have you had COVID-19?			
Yes	11.8 (2)	12.0 (36)	15.5 (17)
No	46.5 (8)	58.8 (177)	62.2 (69)
It is difficult to say unequivocally	41.7 (7)	29.2 (88)	21.8 (24)
Has your body weight changed during the pandemic?			
No	47.1 (8)	47.2 (142)	40.0 (44)
Increased	47.1 (8)	33.9 (102)	41.8 (46)
Decreased	5.8 (1)	18.9 (57)	18.2 (20)
Did you exercise at home during the pandemic?			
Yes	41.2 (7)	48.8 (147)	60.1 (67)*
No	58.8 (10)	51.2 (154)	39.9 (43)
What is a food supplement?			
A preparation that treats nutritional deficiencies	0.0 (0)	2.0 (6)	0.9 (1)
A preparation that supplements nutritional deficiencies	100 (17)	98.0 (295)	99.1 (109)
Do you think food supplements differ from medications?			
Yes	100 (17)	97.0 (292)	99.1 (109)
No	0.0 (0)	0.0 (0)	0.9 (1)
I do not know	0.0 (0)	3.0 (9)	0.0 (0)
What categories of food supplements did you use during the pandemic?#			
Vitamin–mineral supplements	88.2 (15)	79.4 (239)	85.5 (94)
Probiotics	11.8 (2)	22.6 (68)	28.2 (31)
Prebiotics	0.0 (0)	5.0 (15)	5.5 (6)
Supporting immunity	29.4 (5)	45.5 (137)	55.5 (61)
Supporting weight loss	0.0 (0)	2.7 (8)	2.7 (3)
Improving the condition of hair, skin, and nails	35.3 (6)	18.6 (56)	41.8 (46)
Supporting the functioning of the urinary tract	5.9 (1)	3.0 (9)	2.7 (3)
Supporting the heart	5.9 (1)	3.7 (11)	5.5 (6)
Supporting memory	5.9 (1)	6.3 (19)	9.1 (10)
Supporting lowering cholesterol levels	0.0 (0)	2.0 (6)	1.8 (2)
Vision support	5.9 (1)	2.0 (6)	10.0 (11)
Supporting the functioning of the joints	5.9 (1)	5.3 (16)	7.3 (8)
Relieving the symptoms of menopause	0.0 (0)	0.0 (0)	0.9 (1)
Supporting the digestive tract	5.9 (1)	4.3 (13)	6.4 (7)
Improving well-being	0.0 (0)	5.0 (15)	9.1 (10)
Facilitating sedation and sleep	5.9 (1)	11.3 (34)	12.7 (14)
Supporting libido	0.0 (0)	0.7 (2)	1.8 (2)
Supporting alcohol metabolism	0.0 (0)	1.7 (5)	0.9 (1)
For athletes	5.9 (1)	4.3 (13)	6.4 (7)
Removing excess water	0.0 (0)	0.0 (0)	0.0 (0)
Other	0.0 (0)	0.3 (1)	0.0 (0)
I did not use food supplements	0.0 (0)	0.0 (0)	0.0 (0)

Table 4. Cont.

Variable	Only Zinc <i>n</i> = 17 % (n)	Only Vitamin D <i>n</i> = 301 % (n)	Both Food Supplements <i>n</i> = 110
Why did you use such food supplements?#			
Not applicable	0.0 (0)	0.0 (0)	0.0 (0)
To improve health	35.3 (6)	54.8 (165)	54.5 (60)
Due to a pharmacist's recommendation	17.6 (3)	3.0 (9)	5.5 (6)
Due to a doctor's recommendation	17.6 (3)	0.3 (1)	0.0 (0)
To supplement deficiencies of vitamins and minerals	52.9 (9)	72.1 (217)	69.1 (76)
To supplement the therapy prescribed by doctor	0.0 (0)	13.3 (40)	14.5 (16)
Due to a friend's recommendation	5.9 (1)	6.6 (20)	0.9 (1)
Because I was encouraged by TV/media/Internet advertising	0.0 (0)	0.7 (2)	0.9 (1)
Other	0.0 (0)	1.0 (3)	1.8 (2)
What do you usually use to wash down food supplements and medications?#			
Tea	11.8 (2)	13.3 (40)	18.2 (20)
Cola	5.9 (1)	0.6 (2)	1.8 (2)
Not applicable	0.0 (0)	0.0 (0)	0.0 (0)
I do not drink	0.0 (0)	0.0 (0)	6.4 (7)
Juice	11.8 (2)	5.3 (16)	0.0 (0)
Water	94.1 (16)	98.0 (295) ***	97.3 (107)
Coffee	17.6 (3)	3.3 (10)	0.9 (1)
Milk	0.0 (0)	0.0 (0)	0.0 (0)
Other	0.0 (0)	0.0 (0)	0.0 (0)
Do you think there were more advertisements for food supplements during the pandemic?			
No	0.0 (0)	2.7 (8)	0.9 (1)
Yes	35.3 (6)	41.5 (125)	37.3 (41)
I did not notice a change	64.7 (11)	55.8 (168)	61.8 (68)
Do you use food supplements in the amount recommended on the package?			
I do not use it	0.0 (0)	0.0 (0)	0.0 (0)
No, I use lower doses	0.0 (0)	6.3 (19)	7.3 (8)
No, I use higher doses	0.0 (0)	8.3 (25)	13.6 (15)
Yes	100.0 (17) ***	85.4 (257)	79.1 (87)
Do you think food supplements can have side effects?			
No, taking them is absolutely safe	11.8 (2)	10.3 (31)	10.0 (11)
Yes	88.2 (15)	89.7 (270)	90.0 (99)
How do you assess the advisability of using food supplements?			
They should be used only in the event of identified deficiencies	94.1 (16) **	82.1 (247)	83.6 (92)
Their use is unnecessary	5.9 (1)	5.0 (15)	0.9 (1)
I have no opinion	0.0 (0)	12.9 (39)	15.5 (17)
Do you think food supplements can be overdosed on?			
No, they're safe	11.8 (2)	5.3 (16)	7.3 (8)
Yes	88.2 (15)	94.7 (285)	92.7 (102)
Do you think that food supplements can interact with medications prescribed by your doctor, and thus affect the effectiveness of therapy?			
No, they're safe	23.6 (4)	8.0 (24)	10.9 (12)
Yes	76.4 (13)	92.0 (277) **	89.1 (98)
Has the pandemic affected your use of food supplements?			
No	76.5 (13)	73.7 (222)	59.1 (65)
Yes, I use fewer	0.0 (0)	0.7 (2)	1.8 (2)
Yes, I use more	23.5 (4)	25.6 (77)	39.1 (43)
I did not use dietary supplements	0.0 (0)	0.0 (0)	0.0 (0)

Differences between the various pandemic waves: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, # multiple choice question.

High health awareness may be indicated by the fact that people choosing both ingredients in food supplements were also active and exercised at home (60.1%). People who took both zinc and vitamin D also used other vitamin and mineral ingredients (85.5% of the respondents) and other FS affecting immunity (55.5%). It is surprising that a large percentage of the respondents also took preparations supporting the appearance of hair, skin, and nails (41.8%). It should be emphasized that 100% of respondents using zinc took the preparations in accordance with the recommendations, and 94.1% indicated that food supplements should be used only in the case of proven deficiencies (Table 4).

In our research, we found that the highest percentage of people during all three waves used food supplements containing only vitamin D, while searches on Google Trends indicate that, during the first wave, information about zinc was more popular—the importance of vitamin D increased during the second wave (Figure 1).

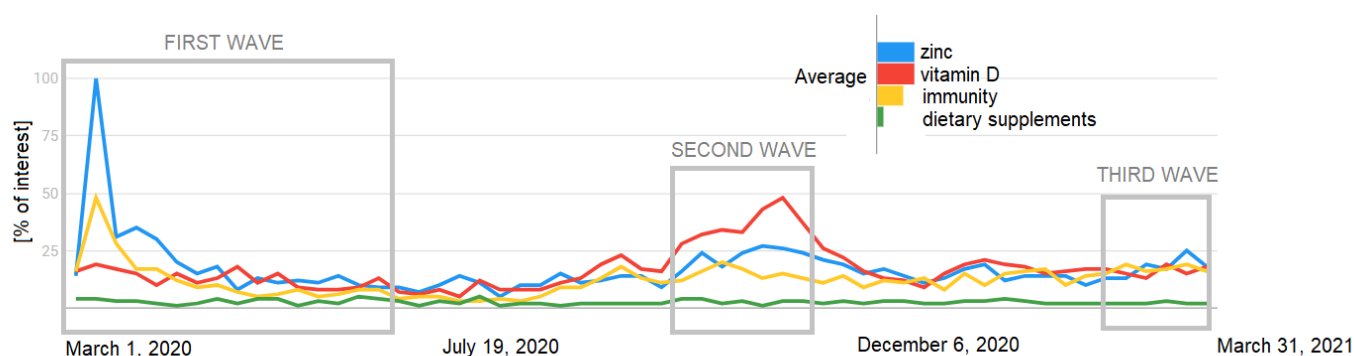


Figure 1. The popularity of searching for selected terms (own design based on data from Google Trends).

4. Discussion

There are several dietary and lifestyle factors that could influence immunity in positive as well as negative ways. The COVID-19 pandemic has prompted people to search for natural methods to boost immunity, including FS usage. Several vaccines are currently approved for human use, but there is still a need to develop effective therapies to treat COVID-19 and alleviate the negative health consequences of the disease.

Currently, there are quite a few studies available on the impact of the pandemic on lifestyles and nutrition. However, there are not many studies dealing with COVID-19 and dietary supplement intake. To the best of our knowledge, our study is the first to assess the consumption of dietary supplements in Poland during the three COVID-19 waves.

A Google Trends analysis showed that, in Poland, in relation to the coronavirus, the following terms were searched for: vitamin C, vitamin D, and *Glycyrrhiza glabra*; globally, there were also search terms such as vitamin K, selenium, zinc, garlic, onion, elderberry, lactoferrin, echinacea, and *Nigella sativa* L. Polish residents were trying to find antiviral properties for turmeric, garlic, and iodine as well as immune-boosting properties for fish oil [14].

Kamarli Altun et al. conducted a cross-sectional study among Turkish dietitians concerning the supplements, functional foods, and herbal medicines they used to protect themselves against SARS-CoV-2 infection. Nearly 90% of the study participants found that proper nutrition could affect the clinical course of the disease and almost all respondents (94.5%) declared FS intake. Less than half of dietitians (46.1%) started using herbal medicine, while nearly one-third included functional foods into the diet (34.9%). Fish oil was the most commonly chosen FS (81.9%). Women were twice as likely to use FS as men [19]. In this study we reported a lower prevalence of FS intake: most of the participants declared usage of FS 57.2%, 80.3%, and 76.1% in the first, second, and third waves, respectively, of the pandemic in Poland. The most often chosen type of FS was preparations with vitamins and minerals. More people reported that pandemic affect on using these supplements in the second wave compared to the first and third waves (23.1% vs. 8.9% and 17.0%).

Another cross-sectional study was carried out by Alfawaz et al. among Saudi Arabian residents, focused on changes in FS usage before the pandemic and during lockdown. Males tended to use FS (multivitamin, selenium, zinc, and vitamin D) more frequently than females. Among the subgroup of COVID-19 patients, men used more multivitamin and zinc supplements than women, while women had a higher intake of supplements with vitamins D and C. The male study participants 26–35 years of age declared a significantly higher use of multivitamin supplements than females (30.1 vs. 22.6%; $p < 0.054$) of the same age group. As determinants of FS usage, researchers distinguished the influence of age, level of education, and income [20].

The supplementation pattern among COVID-19 patients in Teheran was analyzed by Bagheri et al. Significantly higher vitamin D intake was reported in outpatients (30%) compared to hospitalized patients (16.5%). It was observed that vitamin D intake was related to a reduced risk of exacerbation of the disease. Moreover, a relevant difference was found considering zinc intake—9% vs. 2% in outpatients and inpatients, respectively. However, none of the patients declared the usage of multivitamins, vitamin C, vitamin E, folic acid, iron, omega-3, and omega-6 fatty acids [21]. In this research, none of the participants had COVID-19 in the first wave, 11.8% in the second, and 17.6% in the third waves; 14%, 28.3%, and 33.7% of respondents could not equivocally say whether they had SARS-CoV-2 infection. Considering the supplementation of vitamin D and zinc at the beginning of the pandemic, most of the participants (63.6%) did not take them during the first wave, which is contrary to the responses about the second (30.1%) and third (39.4%) waves of the pandemic. This difference was statistically significant. Vitamin D intake was declared by 22.8%, 37.6%, and 32.9%, while zinc was taken by 1.3%, 1.9%, and 2.1%; both compounds were used by 7.2%, 14.0%, and 12.5% of participants during the three waves of COVID-19 in Poland. Our results indicate a higher prevalence of intake of FS with vitamin D and zinc among the Polish population than among the Iranian population.

While in many countries increased interest in diet supplementation was observed, the findings of the cross-sectional study among the Lebanese population showed a decreased supplement intake. Before the pandemic, over 73% of the respondents used FS, while after the COVID-19 outbreak it was 69.9%. However, for specific subgroups of FS, increased intake was reported. Noticeably higher usage of antioxidants (14% vs. 15.6%), vitamin C (35.3% vs. 42.1%), vitamin D (35.5% vs. 41%), vitamin E (15.2% vs. 17.5%), and zinc (18.8% vs. 29.3%) was reported [22]. Our results indicate that the COVID-19 pandemic breakout did not generally influence the pattern of supplementation among Polish residents. The vast majority of the respondents (90.7%, 61.8%, and 81.8% in the three waves, respectively) did not change their FS usage. On the other hand, we found that if the study participants decided to modify something in their diet supplementation, they tended to use more preparations, especially during the second wave (23.1%).

Another analysis considering supplementation patterns during the pandemic was carried out on the basis of the results of the application-based community survey. This study involved 175,652 supplement users and 197,068 nonusers. The risk of COVID-19 infection among women who declared intake of probiotics, omega-3 fatty acids, multivitamins, and vitamin D was lowered by 14%, 12%, 13%, and 9%, respectively. No protective association was observed among men. Moreover, no positive effect was found for respondents taking vitamin C, zinc, or garlic FS [23].

Vitamin D supplementation had a positive influence on recovery from symptoms in patients with mild to moderate COVID-19. Sabico et al., in a randomized control trial, administrated two weeks of oral supplementation of vitamin D (1000 UI vs. 5000 UI) to patients with suboptimal vitamin D status. In the group that received a higher dose, a reduced time to recovery from cough and sensory loss was found. Based on these findings, it seems reasonable to recommend vitamin D as an adjuvant to COVID-19 therapy for patients with mild to moderate symptoms [24].

Vitamin D supplementation and the risk of COVID-19 were assessed in a prospective study by Hao et al. based on data from the UK Biobank cohort study. Habitual use of vitamin D supplements was related to a 34% lower risk of infection [25].

Szarpak et al. carried out a meta-analysis of four studies, comprising 1474 patients, focusing on the influence of zinc on COVID-19 patient outcomes. In the group of patients who received zinc supplementation, survival to hospital discharge was 56.8%, while in the group to which supplementation was not administered it was 75.9%. Moreover, patients who were given supplementation had a higher percentage of in-hospital mortality (22.3% vs. 13.6%) and longer hospital stay (7.7 days vs. 7.2 days). Based on these findings, zinc supplementation does not have a beneficial impact on the abovementioned outcomes [26]. Dubourg et al. observed that median blood Zn levels were significantly lower in COVID-19 patients with poor clinical outcomes in comparison to patients with good clinical outcomes (840 µg/L vs. 970 µg/L). Those results may indicate the importance of Zn supplementation during SARS-CoV-2 infection [27].

A positive correlation was shown between Zn deficiency and COVID-19 cases per million among Asian countries in a retrospective study by Ali et al. The prevalence of Zn deficiency was nearly twice as high among Asians compared to the European population (17.5% vs. 8.9%). On the other hand, a significantly negative correlation between serum Zn levels and COVID-19 deaths per million was recognized among the European population [28]. However, cohort studies are needed to confirm these observations.

In research conducted by Adbelmaksoud et al., Zn supplementation (220 mg of zinc sulfate twice a day) was related a shortened time of smell recovery after SARS-CoV-2 infection, without an influence on the total recovery of the disease. Moreover, serum Zn levels were similar considering subgroups in the case of disease severity or the presence or absence of olfactory and/or gustatory dysfunction [29].

Thomas et al. carried out a randomized control trial among ambulatory patients ($n = 214$) suffering from COVID-19. Patients were randomized in a 1:1:1:1 allocation ratio every 10 days. In the first intervention group, patients were given 50 mg of zinc gluconate, the second 8000 mg of ascorbic acid, the third both, and in the last group a standard treatment regimen was observed. Researchers did not observe a significant difference in secondary outcomes among the studied groups. The results of the study by Thomas et al. do not confirm the assumption of the study by Dubourg et al. [30].

It should be emphasized that the vast majority of respondents took dietary supplements, and not drugs containing zinc and vitamin D. Drugs were used by 5.1% of the respondents during the first wave; during the second wave it was 15.1%, and during the third 13.1%. The respondents themselves noticed the need for supplementation, and the advice of specialists (doctors, pharmacists) was much less frequently cited. Therefore, education on the proper selection of a preparation (the right chemical form, with good digestibility) and the right dose (in accordance with the recommendations corresponding to the daily requirement for supplemented dietary components) seems to be important. It was estimated that many respondents did not provide the names of the zinc and vitamin D preparations used—this may indicate that they do not pay attention to it, while one of the important criteria is the price of FS.

The limitations of this study include the unequal gender proportions (the predominance of women)—if the majority of participants in the study were men, the results could be different. However, greater female participation is a fairly common problem in volunteer-based surveys. Moreover, our survey was a retrospective study, so incorrect recall of information by survey participants may be an important problem.

5. Conclusions

The popularity of dietary supplements, especially vitamin and mineral supplements, is gradually increasing in Poland. During the COVID-19 pandemic, the consumption of dietary supplements containing zinc and vitamin D increased, especially among people with higher education, or medical and paramedical education, which indicates the

increased awareness of this social group regarding pro-health prophylaxis. Due to the nonrestrictive registration procedures of dietary supplements, it seems necessary to educate consumers in terms of the selection of appropriate preparations, proper nutrition, and balanced supplementation.

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Informed Consent Statement: The participants of the study were informed that completing the anonymous questionnaire, which did not contain personally identifiable information, was tantamount to consenting to participation in the study.

Data Availability Statement: Excel spreadsheets with data are available from the authors.

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

Appendix A

Table A1. Questionnaire.

Question	Answer
1. Gender	Female/male
2. Age (year)	
3. Height (cm)	
4. Body weight (kg)	
5. Education level	Primary school/higher/secondary
6. Type of education	Medical and related/nonmedical/not applicable
7. Place of living	Village/City < 150,000 inhabitants/City 150,000–250,000 inhabitants/City > 250,000 inhabitants
8. Subjective assessment of the financial situation of the household	Very good/rather good/average/rather bad/bad
9. Number of people in the household	
10. Professional activity	Unemployed person/person working in office /person working online/student
11. Physical activity	Inactivity (sedentary)/low (occasional exercise, 1–3 times a week)/moderate (1 h of training per day)/high (hard physical work and daily workouts)
12. Voivodeship	
13. How would you rate your health at the beginning of the pandemic in Poland?	Very good/good/medium/poor
14. Have you had COVID-19?	Yes/no
15. Chronic diseases	Hypertension/type 1 diabetes/type 2 diabetes/atherosclerosis/gout/hypothyroidism/overactive thyroid gland/allergy/food intolerances/obesity/insulin resistance/cancer/I am not sick
16. Has your body weight changed during the pandemic?	No/increased/decreased
17. Did you exercise at home during the pandemic?	Yes/no
18. What is a food supplement?	A preparation that treats nutritional deficiencies/preparation, which supplements nutritional deficiencies
19. Do you think food supplements differ from medications?	Yes/no
20. Do you think food supplements differ from medications? If so, please specify how.	
21. What categories of food supplements did you use during the pandemic?	Vitamins and minerals/probiotics/prebiotics/supporting immunity/supporting weight loss/improving the condition of hair, skin, and nails/supporting the functioning of the urinary tract/supporting the heart/supporting memory/supporting lowering cholesterol levels/vision support/supporting the functioning of the joints/relieving the symptoms of menopause/supporting the digestive tract/improving well-being/facilitating sedation and sleep/supporting alcohol metabolism/for athletes/removing excess water/I did not use dietary supplements

Table A1. Cont.

Question	Answer
22. Have you used zinc and vitamin D food supplements since March 2020/September 2020/February 2021?	No/only drugs/yes both/only zinc/only vitamin D
23. Why did you use such food supplements?	To improve health/to supplement deficiencies of vitamins and minerals/to supplement the therapy prescribed by my doctor/because a pharmacist recommended them/because I was encouraged by TV, media, and/or Internet advertising /someone I knew recommended them to me/not applicable
24. The names of all food supplements currently used, together with the name of the manufacturer	Not applicable/someone I knew recommended them to me/to improve health
25. The names of chronic medicines used (name and dose)	
26. Why did you use such food supplements?	
27. What do you usually use to wash down food supplements and medications?	Tea/cola/not applicable/I don't drink/juice/water
28. Do you think there were more advertisements for food supplements during the pandemic?	Yes/no/I didn't notice a change
29. Do you use food supplements in the amount recommended on the package?	I do not use them at all/no, I use lower doses/no, I use higher doses/yes
30. Do you think food supplements can have side effects?	No, taking them is absolutely safe/yes
31. How do you assess the advisability of using food supplements?	They should be used in the event of identified deficiencies/their use is unnecessary/I have no opinion
32. Do you think food supplements can be overdosed on?	No, they're safe/yes
33. Do you think that food supplements can interact with medications prescribed by your doctor, and thus affect the effectiveness of therapy?	No, they're safe/yes
34. Has the pandemic affected your use of food supplements?	No/yes, I use fewer/yes, I use more
35. Have you heard that preparations containing zinc and vitamin D can support immunity and be helpful in the prevention of viral infections?	Yes/no
36. Any additional comments	

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Publikacja H7

FOOD and NUTRITION

MEDICAL UNIVERSITY STUDENTS' PERSPECTIVE ON MARKETING OF DIETARY SUPPLEMENTS

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Abstract: Dietary supplements are a group of products classified as food, prepared in a pharmaceutical form like medicines. This encourages patients to be frequent and even excessive use of these preparations, including thanks to marketing activities. The aim of the study was to assess the opinions of students of the Medical University of Białystok (MUB) on dietary supplements and their marketing. The survey was conducted using the proprietary questionnaire, among 300 students representing all faculties: Faculty of Medicine with the Division of Dentistry and Division of Medical Education In English (FM), Faculty of Pharmacy with the Division of Laboratory Medicine (FP), and Faculty of Health Sciences (FHS). It has been shown that the source of knowledge for FP students is primary classes within their studies (80.6%). Most students of the FHS show a lack of trust in the dietary supplements of their brand pharmacy (89.2%). Students of the MUB are a group of future specialists who, thanks to their knowledge and experience, can recommend dietary supplements that are of good quality, therefore their opinion is important.

Keywords: dietary supplements, marketing, students

Dietary supplements are foods whose purpose is to supplement the diet with deficient ingredients: minerals, vitamins, or other substances. They may have nutritional or other physiological effects, but no therapeutic effect. They appear in concentrated form, among others capsules, tablets, dragees, powder sachets, dropper bottles, liquid ampoules, etc. This form is intended to facilitate dosing (1). The regulation of the Minister of Health contains a list of 13 vitamins and 17 minerals that may be components of dietary supplements. Polish legislation, however, does not define the so-called “other substances”, which allows manufacturers to market newer and newer supplements, which are a response to the created needs of consumers and patients (2).

Supplements are recommended primarily to the following groups of patients: people with vitamin and mineral deficiencies (e.g. during low-energy diets), elderly people, people excluding products or groups of products from the diet (e.g. vegetarians, lactose-intolerant patients), and pregnant women (3). At the same time, market data indicate that Poles willingly reach for dietary supplements – e.g. a study by Matysek-Nawrocka et al. (2016) indicates that 77%

of respondents used dietary supplements. The remaining respondents indicated that they did not use or remember – which may indicate difficulties in distinguishing dietary supplements from drugs (4).

Dietary supplements are not as closely controlled as drugs - in the individual phase of clinical trials. This results in the fact that contaminated or adulterated dietary supplements may be on sale. For example, it was noted, contamination of dietary supplements with mercury (in tablets containing Bamboo shoots and Horsetail: 4212.04 µg/kg, which exceeds the norm at 100 µg/kg, which the authors refer to) (5). This fact does not discourage consumers: it is estimated that in Poland approximately 38.2% of women and 32.1% of men use dietary supplements. The most used categories include magnesium (7.56% of the population), immunostimulants (6.58%), and probiotics (6.13%) (6).

Various types of marketing activities influence consumer's decisions. Their goal is to define and satisfy social needs in order to make a profit (7). One of the main places where dietary supplements are sold are pharmacies. Marketing activities undertaken by managers include: all kinds of promotional

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initiatives, maintaining an appropriate pricing policy, matching the range of products offered, appropriate personnel, and exposing the location of points of sale outlets. The marketing success of the pharmacy depends on the above 6 elements that make up the so-called retail mix (8). Marketing activities seem to be effective because in 2018 pharmacy and non-pharmacy sales of dietary supplements in Poland amounted to PLN 5.4 billion (9).

Therefore, the aim of the study was to assess the knowledge and opinions of students on the marketing of dietary supplements. Medical University students are a specific and important group: they are the ones who will recommend specific preparations to their patients in the future, being the reason why their opinion is extremely important. For the first time, our study presents cross-sectional data, including the views of students from all faculties of MUB.

EXPERIMENTAL

The study was carried out on 300 students of the MUB: from November 13 to December 7, 2020, using the proprietary questionnaire. The questionnaire was verified with a trial test, according to Karbownik et al. (10).

The students filled in an original questionnaire containing 31 questions, which was made available online. Questions 1 to 9 concerned the field of study and year of study, gender, age, height, body weight, subjective assessment of the material situation, health condition, and physical activity. Questions from 10 to 30 concerned the dietary supplements used and the opinions of students, and question 31 – was an open

question, allowing to add own opinion. Sending the completed questionnaire was tantamount to consent to the participation in the survey. For multiple-answer questions (regarding preferences), the sum of the group sizes is greater than 300.

Conducting an anonymous questionnaire did not require the approval of the Bioethics Committee of the Medical University of Białystok.

In order to assess the relationship between the nominal features, the chi-square test of independence was performed. The level of statistical significance was set at $p < 0.05$. Microsoft Excel 2019 and Statistica 13.3 Statsoft were used for data analysis.

RESULTS

The characteristics of the surveyed group are presented in Table 1. The surveyed students ranged in age from 18.0 to 29.0 years, their BMI ranged from 15.79 to 36.78 kg/m².

Among students from the Faculty of Medicine with the Division of Dentistry and Division of Medical Education In English (FM), the largest percentage of respondents were medical students (80.3% of respondents from this Faculty), among students from the Faculty of Pharmacy with the Division of Laboratory Medicine (FP): pharmacy students (45.6%), and of the Faculty of Health Sciences (FHS): dietetics students: 32.4%. The highest percentage of the respondents from among FM was 1st year students, among FP students: 4th year, and FHS students: 5th year (Table 2). Women constituted the greater part of the group: among students with FM, it was 80.3%, with FP: 88.1%, and among

Table 1. Characteristics of the studied group, taking into account the division into faculty.

Faculty Parameters	Faculty of Medicine with the Division of Dentistry and Division of Medical Education In English (FM) (n = 66)	Faculty of Pharmacy with the Division of Laboratory Medicine (FP) (n = 160)	Faculty of Health Sciences (FHS) (n = 74)
Age [years]			
Av ± SD (Min-Max)	22.1 ± 2.1 (19.0-27.0)	22.4 ± 1.9 (19.0-29.0)	21.8 ± 1.8 (18.0-26.0)
Growth [cm]			
Av ± SD (Min-Max)	169.6 ± 7.8 (152.0-186.0)	168.5 ± 7.1 (156.0-190.0)	167.6 ± 8.3 (151.0-195.0)
Weigh [kg]			
Av ± SD (Min-Max)	62.5 ± 11.0 (46.0-96.0)	63.9 ± 13.2 (45.0-130.0)	62.3 ± 10.8 (46.0-95.0)
BMI [kg/m ²]			
Av ± SD (Min-Max)	21.67 ± 3.01 (16.26-32.08)	22.42 ± 3.64 (16.14-36.78)	22.06 ± 2.48 (15.79-27.78)

Av – average, Max – maximum, Min – minimum, n – group size, SD – standard deviation

FHS students: 86.5%, which may also indirectly indicate the gender structure among students of particular fields of study. The majority of respondents assessed their physical activity as low: 69.7%, 75.0%, and 63.5%, respectively. The largest percentage of

students at individual faculties were people who assessed their material situation as good (54.5%, 78.2%, and 85.1%). Similarly, the largest percentage of the respondents as those who described their health as good (74.2%, 63.8%, and 70.3%) (Table 2).

Table 2. Data on the field and year of study of the respondents, their gender, physical activity, and subjective assessment of the material situation and health.

<div>Parameters</div> <div>Faculty</div>	Faculty of Medicine with the Division of Dentistry and Division of Medical Education In English (FM) (n = 66)	Faculty of Pharmacy with the Division of Laboratory Medicine (FP) (n = 160)	Faculty of Health Sciences (FHS) (n = 74)
Courses of study			
Courses	Medicine: 80.3% (n = 53)	Pharmacy: 45.6% (n = 73)	Dietetics: 32.4% (n = 24)
			Physiotherapy: 20.3% (n = 15)
		Cosmetology: 38.1% (n = 61)	Nursing: 17.6% (n = 13)
			Emergency medicine: 6.8% (n = 5)
	Dentistry: 18.2% (n = 12)	Laboratory medicine: 12.5% (n = 20)	Midwifery: 6.8% (n = 5)
			Speech therapy and phonoaudiology: 6.8% (n = 5)
	Dental technology: 1.5% (n = 1)	PhD studies: 3.8% (n = 6)	Electroradiology: 5.4% (n = 4)
			Public health: 3.9% (n =3)
Year of study			
I	22.7% (n = 15)	10.6% (n = 17)	17.5% (n = 13)
II	13.6% (n = 9)	16.3% (n = 26)	16.2% (n = 12)
III	15.3% (n = 10)	6.3% (n = 10)	12.2% (n = 9)
IV	13.6% (n = 9)	29.4% (n = 47)	18.9% (n = 14)
V	19.7% (n = 13)	26.2% (n = 42)	33.8% (n = 25)
VI	12.1% (n = 8)	7.5% (n = 12)	0.0% (n = 0)
Graduate	1.5% (n = 1)	0.0% (n = 0)	0.0% (n = 0)
Intern	1.5% (n = 1)	0.0% (n = 0)	1.4% (n = 1)
PhD	0.0% (n = 0)	3.7% (n = 6)	0.0% (n = 0)
Gender			
Woman	80.3% (n = 53)	88.1% (n = 141)	86.5% (n = 64)
Man	19.7% (n = 13)	11.9% (n = 19)	13.5% (n = 10)
Physical activity			
Low (occasional exercise 1-3 times a week)	69.7% (n = 46)	75.0% (n = 120)	63.5% (n = 47)
Moderate (1 hour of activity per day)	24.2% (n = 16)	21.9% (n = 35)	35.1% (n = 26)
High (heavy physical flare and daily workouts)	6.1% (n = 4)	3.1% (n = 5)	1.4% (n = 1)

Table 2. Data on the field and year of study of the respondents, their gender, physical activity, and subjective assessment of the material situation and health (cont.).

Parameters \ Faculty	Faculty of Medicine with the Division of Dentistry and Division of Medical Education In English (FM) (n = 66)	Faculty of Pharmacy with the Division of Laboratory Medicine (FP) (n = 160)	Faculty of Health Sciences (FHS) (n = 74)
Subjective assessment of the material status			
Very good	40.9% (n = 27)	16.8% (n = 27)	9.5% (n = 7)
Good	54.5% (n = 36)	78.2% (n = 125)	85.1% (n = 63)
Bad	4.6% (n = 3)	5.0% (n = 8)	5.4% (n = 4)
Subjective health assessment			
Very good	21.2% (n = 14)	31.3% (n = 50)	23.0% (n = 17)
Good	74.2% (n = 49)	63.8% (n = 102)	70.3% (n = 52)
Bad	4.6% (n = 3)	4.9% (n = 8)	6.7% (n = 5)

n – group size

Table 3 presents data on the opinions and experiences of students with regard to dietary supplements. Three divisions of the studied group were taken into account: in terms of faculty, gender, and period of study. Statistical analyzes were performed within the above groups.

Opinions are divided as to what a dietary supplement is: about three-quarters of students stated that it is a food aimed at supplementing nutritional deficiencies (FM: 77.3%, FP: 77.5%, FHS: 70.3%). Significantly more men answered this question correctly (85.7%).

Table 3. Dietary supplements in the opinion of students of three faculties of the Medical University in Białystok.

Parameters \ Division	Faculty			Gender		Period of study	
Parameters \ Faculty	% of FM students (n = 66)	% of FP students (n = 160)	% of FHS students (n = 74)	% of women (W) (n = 258)	% of men (M) (n = 42)	% of students on I-III years (I group) (n = 121)	% of students of subsequent years of study (II group) (n = 179)
Question: What is a dietary supplement?							
A drug, a preparation that treats nutritional deficiencies	22.7% (n = 15)	22.5% (n = 36)	29.7% (n = 22)	26.0% (n = 67)	14.3% (n = 6)	24.8% (n = 30)	24.0% (n = 43)
It is food, it supplements nutritional deficiencies	77.3% (n = 51)	77.5% (n = 124)	70.3% (n = 52)	74.0% (n = 191)	85.7% (n = 36)*	75.2% (n = 91)	76.0% (n = 136)
Question: What do you primarily follow when purchasing a dietary supplement?							
Price	40.9% (n = 27)	37.5% (n = 60)	43.2% (n = 32)	39.9% (n = 103)	38.1% (n = 16)	42.1% (n = 51)	38.0% (n = 68)
Composition (chemical form)	77.3% (n = 51)	90.0% (n = 128)*	71.6% (n = 53)	77.5% (n = 200)	76.2% (n = 32)	69.4% (n = 84)	82.7% (n = 148)
Habit	7.6% (n = 5)	7.5% (n = 12)	8.1% (n = 6)	7.8% (n = 20)	7.1% (n = 3)	8.3% (n = 10)	7.3% (n = 13)
Advertisement	0.0% (n = 0)	2.5% (n = 4)	6.8% (n = 5)	3.5% (n = 9)	0.0% (n = 0)	3.3% (n = 4)	2.8% (n = 5)
Pharmacist's recommendation	39.4% (n = 26)	43.8% (n = 70)	24.3% (n = 18)	40.3% (n = 104)*	23.9% (n = 10)	42.1% (n = 51)	35.2% (n = 63)
Brand/company	3.0% (n = 2)	16.9% (n = 27)	8.1% (n = 6)	11.2% (n = 29)	14.3% (n = 6)	5.0% (n = 6)	16.2% (n = 29)
Doctor's recommendation	39.4% (n = 26)	20.6% (n = 33)	31.1% (n = 23)	29.5% (n = 76)	14.3% (n = 6)	28.1% (n = 34)	26.8% (n = 48)

Table 3. Dietary supplements in the opinion of students of three faculties of the Medical University in Białystok (cont.).

Division	Faculty			Gender		Period of study	
Parameters \ Faculty	% of FM students (n = 66)	% of FP students (n = 160)	% of FHS students (n = 74)	% of women (W) (n = 258)	% of men (M) (n = 42)	% of students on I-III years (I group) (n = 121)	% of students of subsequent years of study (II group) (n = 179)
Packaging	1.5% (n = 1)	1.3% (n = 2)	2.7% (n = 2)	1.6% (n = 4)	2.4% (n = 1)	2.5% (n = 3)	1.1% (n = 2)
Another reason - total: - the highest price, - recommendation by someone (e.g. on a blog), - dose, - recommendation of a dietitian, - drug status, not supplement status, - quality tests, - needs and knowledge, - place of purchase: pharmacy, - lecturer's recommendation	3.0% (n = 2)	5.0% (n = 8)	4.2% (n = 3)	4.0% (n = 10)	7.2% (n = 3)	3.2% (n = 5)	4.6% (n = 8)
Does not take supplements	6.0% (n = 4)	1.9% (n = 3)	1.4% (n = 1)	1.6% (n = 4)	9.5% (n = 4)	2.5% (n = 3)	2.8% (n = 5)
Question: The main sources of knowledge about dietary supplements							
Internet	66.7% (n = 44)	65.6% (n = 105)	73.0% (n = 54)	65.5% (n = 169)	81.0% (n = 34)	65.3% (n = 79)	69.3% (n = 124)
TV commercials	9.1% (n = 6)	8.1% (n = 13)	8.1% (n = 6)	8.5% (n = 22)	7.1% (n = 3)	12.4% (n = 15)	5.6% (n = 10)
Professional press	24.2% (n = 16)	18.1% (n = 29)	17.6% (n = 13)	19.0% (n = 49)	21.4% (n = 9)	14.0% (n = 17)	22.9% (n = 41)
Friends and family	25.8% (n = 17)	14.4% (n = 23)	14.9% (n = 11)	17.8% (n = 46)	11.9% (n = 5)	25.6% (n = 31)	11.2% (n = 20)
Studies	57.6% (n = 38)	80.6% (n = 129)**	43.2% (n = 32)	66.7% (n = 172)	64.3% (n = 27)	51.2% (n = 62)	76.5% (n = 137)**
Pharmacist	37.9% (n = 25)	41.3% (n = 66)	20.3% (n = 15)	37.2% (n = 96)	23.8% (n = 10)	43.0% (n = 52)	30.2% (n = 54)
Doctor	42.4% (n = 28)	19.4% (n = 31)	18.9% (n = 14)	26.0% (n = 67)	14.3% (n = 6)	31.4% (n = 38)	19.6% (n = 35)
Question: Have you noticed a beneficial effect of using supplements?							
Yes	56.1% (n = 37)	54.4% (n = 87)	55.4% (n = 41)	56.9% (n = 146)	45.2% (n = 19)	54.5% (n = 66)	55.3% (n = 99)
No	25.8% (n = 17)	16.2% (n = 26)	25.7% (n = 19)	20.2% (n = 52)	23.8% (n = 10)	22.3% (n = 27)	19.6% (n = 35)
Not applicable	18.1% (n = 12)	29.4% (n = 47)	18.9% (n = 14)	22.9% (n = 60)	31.0% (n = 13)	23.2% (n = 28)	25.1% (n = 45)
Question: Have you noticed any side effects of dietary supplements?							
Yes	4.5% (n = 3)	3.7% (n = 6)	2.7% (n = 2)	3.5% (n = 9)	4.8% (n = 2)	1.7% (n = 2)	5.0% (n = 9)
No	78.8% (n = 52)	70.0% (n = 112)	78.4% (n = 58)	75.6% (n = 195)	64.3% (n = 27)	78.5% (n = 95)	70.9% (n = 127)
Not applicable	16.7% (n = 11)	26.3% (n = 42)	18.9% (n = 14)	20.9% (n = 54)	30.9% (n = 13)	19.8% (n = 24)	24.1% (n = 43)

Table 3. Dietary supplements in the opinion of students of three faculties of the Medical University in Białystok (cont.).

Division	Faculty			Gender		Period of study		
Parameters	Faculty	% of FM students (n = 66)	% of FP students (n = 160)	% of FHS students (n = 74)	% of women (W) (n = 258)	% of men (M) (n = 42)	% of students on I-III years (I group) (n = 121)	% of students of subsequent years of study (II group) (n = 179)
Question: Do you regularly use dietary supplements?								
Yes	56.1% (n = 37)	39.4% (n = 63)	51.4% (n = 38)	47.3% (n = 122)	38.1% (n = 16)	48.8% (n = 59)	44.1% (n = 79)	
No	36.4% (n = 24)	43.7% (n = 70)	33.8% (n = 25)	39.5% (n = 102)	40.5% (n = 17)	36.4% (n = 44)	41.9% (n = 75)	
Not applicable	7.5% (n = 5)	16.9% (n = 27)	14.8% (n = 11)	13.2% (n = 34)	21.4% (n = 9)	14.8% (n = 18)	14.0% (n = 25)	
Question: How much PLN per month do you spend on dietary supplements?								
Less than PLN 10	6.1% (n = 4)	2.5% (n = 4)	14.7% (n = 11)	2.7% (n = 7)	28.6% (n = 12)	5.0% (n = 6)	7.3% (n = 13)	
10-19	30.3% (n = 20)	11.9% (n = 19)	0.0% (n = 0)	13.5% (n = 35)	9.5% (n = 4)	17.4% (n = 21)	10.1% (n = 18)	
20-40	27.3% (n = 18)	32.5% (n = 52)	35.1% (n = 26)	37.2% (n = 96)	0.0% (n = 0)	35.5% (n = 43)	29.6% (n = 53)	
41-100	3.0% (n = 2)	11.3% (n = 18)	12.2% (n = 9)	10.5% (n = 27)	4.8% (n = 2)	7.4% (n = 9)	11.2% (n = 20)	
above 100	3.0% (n = 2)	0.6% (n = 1)	2.7% (n = 2)	1.6% (n = 4)	2.4% (n = 1)	0.8% (n = 1)	2.2% (n = 4)	
I do not know	4.5% (n = 3)	0.6% (n = 1)	2.7% (n = 2)	1.2% (n = 3)	7.1% (n = 3)	2.5% (n = 3)	1.6% (n = 3)	
Not applicable	25.8% (n = 17)	40.6% (n = 65)***	32.6% (n = 24)	33.3% (n = 86)	47.6% (n = 20)	31.4% (n = 38)	38.0% (n = 68)	
Question: Where do you buy dietary supplements?								
At the pharmacy	80.3% (n = 53)	86.9% (n = 139)	87.8% (n = 65)	88.0% (n = 227)	71.4% (n = 30)	87.6% (n = 106)	84.4% (n = 151)	
In a store / cosmetic store	10.6% (n = 7)	5.0% (n = 8)	9.5% (n = 7)	7.0% (n = 18)	9.5% (n = 4)	6.6% (n = 8)	7.8% (n = 14)	
In the herbal and medical store	3.0% (n = 2)	7.5% (n = 12)	5.4% (n = 4)	6.9% (n = 17)	2.4% (n = 1)	7.4% (n = 9)	5.0% (n = 9)	
Over the Internet	19.7% (n = 13)	25.6% (n = 41)	31.1% (n = 23)	25.6% (n = 66)	26.2% (n = 11)	19.8% (n = 24)	29.6% (n = 53)	
Direct sales	1.5% (n = 1)	0.6% (n = 1)	1.4% (n = 1)	0.8% (n = 2)	2.4% (n = 1)	0.8% (n = 1)	1.1% (n = 2)	
Another answer: online pharmacy	1.5% (n = 1)	0.0% (n = 0)	0.0% (n = 0)	0.4% (n = 1)	0.0% (n = 0)	0.0% (n = 0)	0.6% (n = 1)	
Not applicable	9.1% (n = 6)	5.6% (n = 9)	2.7% (n = 2)	4.3% (n = 11)	14.3% (n = 6)	5.0% (n = 6)	6.1% (n = 11)	
Question: What form of dietary supplements do you choose most often?								
Tablets	75.8% (n = 50)	77.5% (n = 124)	81.1% (n = 60)	81.4% (n = 210)	57.1% (n = 24)	80.2% (n = 97)	76.5% (n = 137)	
Capsules	54.5% (n = 36)	60.0% (n = 96)	56.8% (n = 42)	58.1% (n = 150)	57.1% (n = 24)	57.0% (n = 69)	58.7% (n = 105)	
Effervescent tablets	13.6% (n = 9)	12.5% (n = 20)	12.2% (n = 9)	12.0% (n = 31)	16.7% (n = 7)	17.4% (n = 21)	9.5% (n = 17)	
Dissolving sachets	7.6% (n = 5)	6.3% (n = 10)	14.9% (n = 11)	7.8% (n = 20)	14.3% (n = 6)	8.3% (n = 10)	8.9% (n = 16)	

Table 3. Dietary supplements in the opinion of students of three faculties of the Medical University in Białystok (cont.).

Division	Faculty			Gender		Period of study	
Parameters	% of FM students (n = 66)	% of FP students (n = 160)	% of FHS students (n = 74)	% of women (W) (n = 258)	% of men (M) (n = 42)	% of students on I-III years (I group) (n = 121)	% of students of subsequent years of study (II group) (n = 179)
Spray	0.0% (n = 0)	0.6% (n = 1)	0.0% (n = 0)	0.4% (n = 1)	0.0% (n = 0)	0.8% (n = 1)	0.0% (n = 0)
Drops	1.5% (n = 1)	8.8% (n = 14)	2.7% (n = 2)	6.2% (n = 16)	2.4% (n = 1)	2.5% (n = 3)	7.8% (n = 14)
Lozenges	1.5% (n = 1)	3.1% (n = 5)	1.4% (n = 1)	2.3% (n = 6)	2.4% (n = 1)	5.0% (n = 6)	0.6% (n = 1)
Syrups	1.5% (n = 1)	1.3% (n = 2)	0.0% (n = 0)	1.2% (n = 3)	0.0% (n = 0)	0.8% (n = 1)	1.1% (n = 2)
Lollipops	0.0% (n = 0)	0.0% (n = 0)	1.4% (n = 1)	0.4% (n = 1)	0.0% (n = 0)	0.8% (n = 1)	0.0% (n = 0)
Jelly beans	0.0% (n = 0)	1.3% (n = 2)	1.4% (n = 1)	1.2% (n = 3)	0.0% (n = 0)	2.5% (n = 3)	0.0% (n = 0)
Other answer - total: - liquid, - Whey protein concentrate	3.0% (n = 2)	0.0% (n = 0)	0.0% (n = 0)	0.4% (n = 1)	2.4% (n = 1)	0.0% (n = 0)	1.2% (n = 2)
Not applicable	7.6% (n = 5)	3.1% (n = 5)	2.7% (n = 2)	3.1% (n = 8)	9.5% (n = 4)	3.3% (n = 4)	4.7% (n = 8)
Question: If the dietary supplement you use is not available, do you buy any substitute?							
Yes	60.6% (n = 40)**	40.0% (n = 64)	35.1% (n = 26)	44.6% (n = 115)**	35.7% (n = 15)	52.9% (n = 64)*	36.9% (n = 66)
No	24.2% (n = 16)	34.4% (n = 55)	47.3% (n = 35)	37.2% (n = 96)	23.1% (n = 10)	31.4% (n = 38)	38.0% (n = 68)
Not applicable	15.2% (n = 10)	25.6% (n = 41)	17.6% (n = 13)	18.2% (n = 47)	41.2% (n = 17)	15.7% (n = 19)	25.1% (n = 45)
Question: In your opinion, are dietary supplements tested before being placed on the market?							
Yes	40.9% (n = 27)	38.8% (n = 62)	54.0% (n = 40)	42.6% (n = 110)	45.2% (n = 19)	62.0% (n = 75)	30.2% (n = 54)
No	59.1% (n = 39)	61.2% (n = 98)	46.0% (n = 34)	57.4% (n = 148)	54.8% (n = 23)	38.0% (n = 46)	69.8% (n = 125)***

FHS - Faculty of Health Sciences, FM – Faculty of Medicine with the Division of Dentistry and Division of Medical Education in English, FP – Faculty of Pharmacy with the Division of Laboratory Medicine, *p < 0.05, **p < 0.01, ***p < 0.001

The factors that guide the respondents when choosing a dietary supplement were analyzed. One of the most important criteria is composition (77.3%, 90.0%, and 71.6% of students respectively). The second important factor is the price (40.9%, 37.5%, and 43.2%). The recommendations of pharmacists (39.4%, 43.8%, and 24.3%) and doctors (39.4%, 20.6%, and 31.1%) are also important for students. Interestingly, advertising influences the purchasing decisions of a very small group of students (0.0%, 2.5%, and 6.8%). Moreover, significant differences were demonstrated in the criteria that men and women follow when making decisions about the purchase of dietary supplements. Representatives of both sexes agreed on such criteria as price (39.9% vs. 38.1%) and composition

(77.5% vs. 76.2%), but women listened to pharmacists' recommendations significantly more often (40.3% vs. 23.9%).

Significant differences were also shown in the case of the source of knowledge about dietary supplements. The most important source is the Internet (indicated by 66.7%, 65.6%, and 73.0% of students). In the case of students of the MUB, studies are the second most important source of knowledge (FM: 57.6% of the respondents, FP: 80.6%, FHS: 43.2%). Pharmacists and doctors are also an important source of knowledge (FM: 37.9%, and 42.4%, FP: 41.3%, and 19.4%, PHS: 20.3%, and 18.9%). Men more often indicated the Internet as the main source of knowledge about dietary supplements (81.0% vs. 65.5%), however, the percentage of people indicating that

studying was one of the main sources of knowledge was similar for both gender (W: 66.7% vs. M: 64.3%). Students with more experience (4th year and higher) significantly more often indicated classes during their studies as the greatest source of knowledge about dietary supplements (76.5% vs. 51.2%).

A beneficial effect resulting from the use of dietary supplements was noted by slightly more than half of the respondents (FM: 56.1%, FP: 54.4%, FHS: 55.4%). Side effects resulting from the use of dietary supplements were reported only by a small group of respondents (FM: 4.5%, FP: 3.7%, FHS: 2.7%), while the regular use of dietary supplements was declared by approximately half of the respondents (FM: 56.1%, FP: 39.4%, FHS: 51.4%). Women more often reported beneficial effects resulting from the use of dietary supplements (W: 56.9% vs. M: 45.2%), but it was men who reported side effects more often (M: 4.8% vs. 3.5%). Almost half of the women declared regular use of dietary supplements (W: 47.3% vs. M: 38.1%).

Among students with FM, students spend an average of PLN 10 to 19 (30.3%) or PLN 20 to 40 (27.3%) per month. The highest percentage of FP students spends between PLN 20 and PLN 40 (32.5%), similar to FHS students (35.1%). It has been shown that pharmacies are the main place to buy dietary supplements (FM: 80.3%, FP: 86.9%, FHS: 87.8%). The second and most frequently indicated place of purchase is the Internet (FM: 19.7%, FP: 25.6%, FHS: 31.1%), therefore dietary supplements in the non-pharmacy trade should also be of good quality.

Students most often buy dietary supplements in the form of tablets and capsules (respectively: 75.8% and 54.5% of students with FM, 77.5% and 60.0% with FP, 81.1%, and 56.8% with FHS).

Significant differences were indicated by analyzing the answers to the question regarding the purchase of a substitute if the preparation the student wants to buy is not available for sale. Most students with FM choose a substitute (60.6%), in the case of students with FP and FHS it is a smaller percentage (40.0% and 35.1%), which indicates attachment to the dietary supplements used. In the absence of a specific dietary supplement on sale, preparations of the same composition are used significantly more often by women than men (44.6%) and significantly more often by students of the initial period of study (52.9%).

The attachment to the use of specific dietary supplements may result from the belief that some of them are tested before being marketed. It has been shown that the highest percentage of FHS students say that dietary supplements are tested (54.0%). Only 40.9% of FM students and 38.8% of FP students were of a similar opinion. Moreover, it was shown that significantly more students of older years of studies (IV year and above, 69.8%) assess that dietary supplements are not tested before they are put on sale.

Table 4 presents data on students' opinions on marketing activities. Students named TV commercials as having the greatest chance of reaching their audience (FM: 72.7%, FP: 78.8%, and FHS: 85.1%). Online advertising came second (FM: 51.5%, FP: 57.5%, FHS: 60.8%). Most of the students judged

Table 4. Students' opinions on the marketing of dietary supplements.

Division	Faculty			Gender		Period of study	
Parameters	% of FM students (n = 66)	% of FP students (n = 160)	% of FHS students (n = 74)	% of women (W) (n = 258)	% of men (M) (n = 42)	% of students on I-III years (I group) (n = 121)	% of students of subsequent years of study (II group) (n = 179)
Question: Which ads do you think have the best chance of reaching audience?							
On the Internet	51.5% (n = 34)	57.5% (n = 92)	60.8% (n = 45)	57.0% (n = 147)	57.1% (n = 24)	59.5% (n = 72)	55.3% (n = 99)
In the press	1.5% (n = 1)	1.3% (n = 2)	1.4% (n = 1)	1.6% (n = 4)	0.0% (n = 0)	0.8% (n = 1)	1.7% (n = 3)
In TV	72.7% (n = 48)	78.8% (n = 126)	85.1% (n = 63)	79.8% (n = 206)	73.8% (n = 31)	82.6% (n = 100)	76.5% (n = 137)
Question: In your opinion, is the number of advertisements for dietary supplements adequate?							
Yes, I like watching supplement ads and hearing about new preparations	3.0% (n = 2)	6.9% (n = 11)	12.2% (n = 9)	7.0% (n = 18)	9.5% (n = 4)	7.4% (n = 9)	7.3% (n = 13)
No, there are far too many of them	97.0% (n = 64)	93.1% (n = 149)	87.8% (n = 65)	93.0% (n = 240)	90.5% (n = 38)	92.6% (n = 112)	92.7% (n = 166)

Table 4. Students' opinions on the marketing of dietary supplements (cont.).

Division	Faculty			Gender		Period of study	
Parameters	% of FM students (n = 66)	% of FP students (n = 160)	% of FHS students (n = 74)	% of women (W) (n = 258)	% of men (M) (n = 42)	% of students on I-III years (I group) (n = 121)	% of students of subsequent years of study (II group) (n = 179)
Question: Do you use dietary supplements from own pharmacy brand?							
Yes	25.8% (n = 17)	16.3% (n = 26)	10.8% (n = 8)	17.4% (n = 45)	14.3% (n = 6)	20.6% (n = 25)	14.5% (n = 26)
No	74.2% (n = 49)	83.7% (n = 134)	89.2% (n = 66)*	82.6% (n = 213)	85.7% (n = 36)	79.4% (n = 96)	85.5% (n = 153)
Question: Do you buy dietary supplements offered under direct marketing?							
Yes	3.0% (n = 2)	6.3% (n = 10)	4.1% (n = 3)	5.4% (n = 14)	2.4% (n = 1)	5.8% (n = 7)	4.5% (n = 8)
No	97.0% (n = 64)	91.8% (n = 147)	95.9% (n = 71)	93.4% (n = 241)	97.6% (n = 41)	93.4% (n = 113)	94.4% (n = 169)
I sell this type of preparations myself	0.0% (n = 0)	1.9% (n = 3)	0.0% (n = 0)	1.2% (n = 3)	0.0% (n = 0)	0.8% (n = 1)	1.1% (n = 2)
Question: Which preparation will you choose (taking into account only the price and advertising criteria)?							
Cheaper but unknown	66.7% (n = 44)	61.3% (n = 98)	73.0% (n = 54)	67.1% (n = 173)	54.8% (n = 23)	62.8% (n = 76)	67.0% (n = 120)
More expensive but advertised	33.3% (n = 22)	38.7% (n = 62)	27.0% (n = 20)	32.9% (n = 85)	45.2% (n = 19)	37.2% (n = 45)	33.0% (n = 59)
Question: What is, in your opinion, the most effective promotional activity?							
Word of mouth marketing	45.5% (n = 30)	51.3% (n = 82)	43.2% (n = 32)	48.1% (n = 124)	47.6% (n = 20)	46.3% (n = 56)	49.2% (n = 88)
Advertising on television	57.6% (n = 38)	63.8% (n = 102)	58.1% (n = 43)	62.4% (n = 161)	52.4% (n = 22)	62.8% (n = 76)	59.8% (n = 107)
Internet advertising	37.9% (n = 25)	56.9% (n = 91)	48.6% (n = 36)	49.6% (n = 128)	57.1% (n = 24)	52.0% (n = 63)	49.7% (n = 89)
Advertising in the press	1.5% (n = 1)	4.4% (n = 7)	2.7% (n = 2)	3.5% (n = 9)	2.4% (n = 1)	0.0% (n = 0)	5.6% (n = 10)
Other answer - total: - radio advertising, - doctor, - good product quality and competitive price, - advertising in social media and by influencers, - free samples, - I have no opinion.	10.6% (n = 7)	3.7% (n = 5)	1.4% (n = 1)	5.1% (n = 13)	0.0% (n = 0)	2.5% (n = 3)	5.6% (n = 10)

FHS - Faculty of Health Sciences, FM - Faculty of Medicine with the Division of Dentistry and Division of Medical Education in English, FP - Faculty of Pharmacy with the Division of Laboratory Medicine, * p < 0.05

the number of ads too high (FM: 97.0%, FP: 93.1%, FHS: 87.8%). Moreover, it was shown that a significantly higher percentage of FHS students do not use their own pharmacy brand dietary supplements (89.2%) compared to 83.7% of FP students and 74.2% of FM students.

Despite the fact that most students previously indicated that the composition of supplements is the most important, about two-thirds of respondents

indicated that they would choose a cheaper but unknown product (FM: 66.7%, FP: 61.3%, FHS: 73.0%). The students indicated that the most effective marketing activities are: TV advertising (FM: 57.6%, FP: 63.8%, FHS: 58.1%), Internet advertising (FM: 37.9%, FP: 56.9%, FHS: 48.6%) and word of mouth marketing (FM: 45.5%, FP: 51.3%, FHS: 43.2%). The lack of belief in the effectiveness of supplements of own brand of pharmacies

is indicated by a similar percentage of representatives of both sexes: over 80% of respondents (W: 82.6% vs. M: 85.7%), while over 90% do not buy dietary supplements offered under direct marketing (W: 93.4% vs. M: 97.6%). Women more often indicate that they would choose a cheaper but unknown supplement (67.1% vs. 54.8%). Women most often indicated TV advertisements as the most effective marketing activity (62.4%), while men: Internet advertising (57.1%).

Early years students more often indicated that television and Internet advertisements had a greater chance of reaching the audience (I: 82.6% and 59.5% vs. II: 76.5% and 55.3%). Both groups of students agreed that in their opinion there were too many advertisements for dietary supplements (I: 92.6% vs. II: 92.7%). Older students are characterized by a greater lack of trust in the dietary supplements of their own brands of pharmacies (II: 85.5% vs. I: 79.4%). Over time, the percentage of people who use dietary supplements sold under direct marketing also decreases

(I: 5.8% vs. II: 4.5%). However, older students slightly more often indicate that they would buy a dietary supplement that is cheaper, but unknown (II: 67.0% vs. 62.8%).

Moreover, it was shown that MUB students most often used dietary supplements containing vitamin D and / or vitamin K (as much as 35.33%). The second most frequently chosen type of dietary supplements were preparations containing omega 3 and / or omega 6 and / or omega 9. In addition, 4% of students used supplements with zinc and 3.67% dietary supplements affecting the immunity (e.g. containing rutin) (Figure 1).

DISCUSSION AND CONCLUSION

The opinion of medical university students may result in the recommendation of specific dietary supplements for patients in future work or the lack of it, therefore the purpose of the analyzes was to evaluate

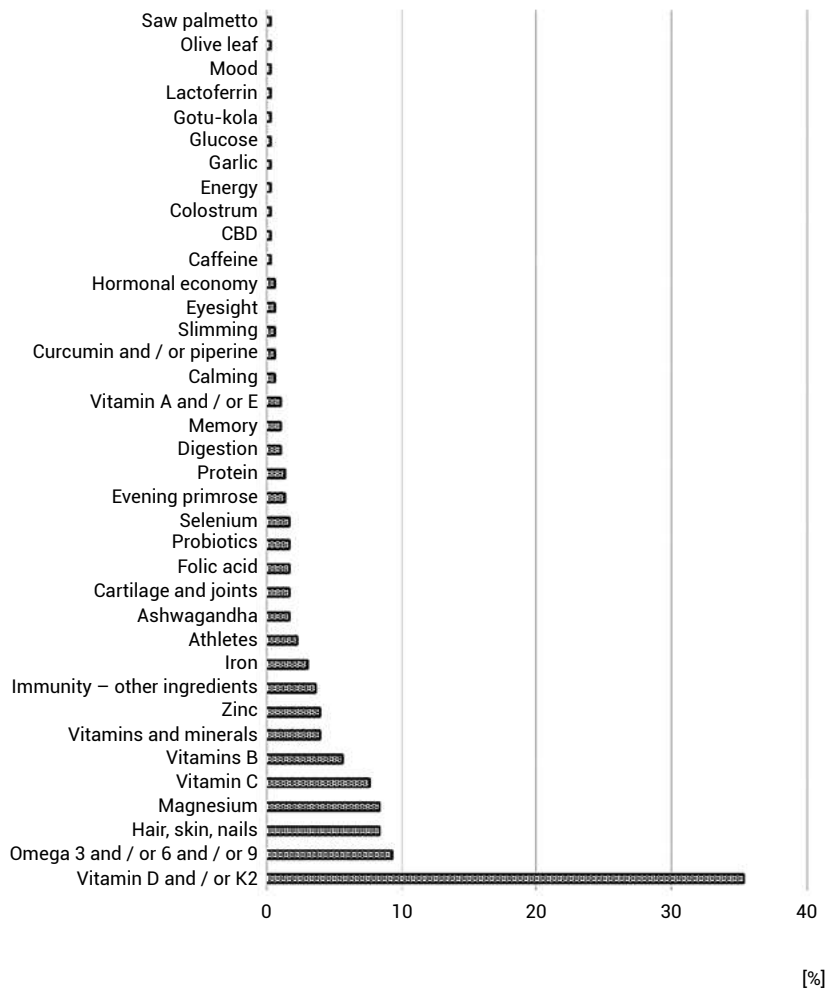


Figure 1. Ingredients or categories of dietary supplements used by students during this study (XI-XII 2020).

these opinions on dietary supplements and marketing activities in this area.

This study showed that 56.1% of FM students, 39.4% of FP students, and 51.4% of FHS students declare regular use of dietary supplements. An analysis broken down by gender allowed to state that 47.3% of women and 38.1% of men declare regular use of dietary supplements. The results obtained by us are higher than those described by Sigłowa et al. (2009), who reported the consumption of dietary supplements by 38.3% of female students and 37.7% of male students (11).

Our earlier study, which aimed, *inter alia*, at the evaluation of the consumption of minerals along with the diet showed that 66% of 79 students of three fields of study (medical analytics, dietetics, and cosmetology) declared the use of dietary supplements during the last year. The most frequently used preparations were dietary supplements from the vitamin and mineral category (12).

Wilczewska et al. (2015), who assessed the knowledge of dietary supplements among selected students of the Medical University in Białystok and among students of post-secondary school, showed that there is a need for increased education in the field of dietary supplements. The authors showed that 21.9% of respondents used dietary supplements during the survey, and 53.7% used them earlier. The most frequently used group of supplements were vitamins and minerals (28.7%). Surprisingly, as many as 39.4% of respondents, when asked about understanding the meaning of the term “dietary supplement”, answered that it is difficult to say (13). In our study, when asked what a dietary supplement is, the correct answer was given by 77.3% of FM students, 77.5% of FP students, and 70.3% of FHS students.

Interesting research results, regarding the opinions of 153 pharmaceutical technicians, pharmacists, and doctors of pharmaceutical sciences, were published by Ratajczak et al. (2019). The opinions of this group of specialists were assessed on dietary supplements that improve mood and the factors that determine the purchase. It was shown that, in the opinion of these specialists, the most important factor determining the choice of a dietary supplement by patients is advertising (91%), specialist advice (80%), and the opinion of friends and family (64%). The information on internet forums (20%) influences the decision to a much lesser extent, and what is worrying, only 12% of the composition and properties. The respondents also assessed that, according to patients, the most important features determining the purchase of a dietary supplement are the price (93%) and the appearance of the packaging (80%). These data indicate

that the opinions of working people are more skeptical than of students (14).

Morris and Avorn (2003) evaluated internet marketing efforts for 8 herbal supplements in 5 popular web browsers. It was estimated that as many as 55% of websites contained information suggesting that supplements cure or help diagnose diseases (15). The information on internet portals must be true, as it is one of the most important media sources for students and patients.

Krejpcio et al. (2011) studied the use of dietary supplements by students of dietetics and psychology. They showed that 61% of dietetics students and 47% of psychology students declare taking dietary supplements, of which 15% of dietetics students and 15% of psychology students taking them every day. In our study, regular use of supplements was declared by 56.1% of FM students, 39.4% of FP students, and 51.4% of FHS students. Interestingly, only 12% of dietetics students and 15% of psychology students consulted a doctor about the consumption of dietary supplements (16).

Among the socio-demographic factors that affect the choice of a vitamin and mineral dietary supplement by adolescents up to 20 years of age, it is indicated, *inter alia*, higher level of mother's education and modification of the diet to make better nutritional decisions (17).

Our research showed that students of all three faculties buy dietary supplements primarily from a pharmacy: 80.3% of FM students, 86.9% of FP students, and 87.8% of FHS students. Only 3.0% of FM students, 7.5% of FP students, and 5.4% of FHS students buy dietary supplements in herbal and medical stores. The place of purchase is very important as it has been shown that in herbal and medical stores it is possible to obtain insufficient information about the products purchased (18, 19). It was estimated that in health food stores unscientific advice was received in 88% of cases, while in pharmacies - only in 27% of cases (19). Research published 5 years ago by Kowalik et al. (2016) showed a similar tendency among students to places where they buy dietary supplements: 85% of students indicated a pharmacy. Our research showed a different trend in purchasing dietary supplements: over the Internet (it was respectively: 19.7% of FM students, 25.6% of FP students, and 31.1% of FHS students, while the Kowalik study showed 10 times fewer students who use this form (20).

In order to increase profits from the sale of dietary supplements, pharmacies, herbal and medical stores, and among others, manufacturers implement various marketing activities. One of the main

types of marketing is internet marketing. Reports are showing that websites do not contain all the necessary information about dietary supplements. For example, Bjelica et al. (2020) analyzed marketing activities regarding dietary supplements that protect the heart. The first 50 pages found by the search engines Google, Bing, and Yahoo were analyzed. It has been shown that the most frequently registered cardioprotective dietary supplements on the Internet were dietary supplements containing omega-3 acids (present on 64.05% of websites) (21). Importantly, information on side effects that may occur was indicated only on 1 website, which constituted 1.75% of the pages finally analyzed (in total: 89). Among the surveyed students whose responses were analyzed in our study, it was shown that side effects after dietary supplements occurred in 4.5% of students of FM, 3.7% of students of FP, and 2.7% of students of FHS (Table 3). Information on potential side effects should be available on websites, in particular on the websites of pharmacies or herbal and medical stores. It is also important from the perspective of patients who use polypharmacy and drugs may interact with dietary supplements.

Research conducted by Ciszek and Duma shows that as many as 71.5% of students declare the use of dietary supplements. About half of this group (36.0%) know the potential dangers of using them (22). Our studies showed that 3.5% of women and 4.8% of men reported side effects resulting from the use of dietary supplements, while the beneficial effects were noticed by 56.9% of women and 45.2% of the surveyed men.

Research conducted by Krejpcio et al. (2011) in a group of 50 physically active people aged 21 to 31 showed that only 40% of respondents knew the definition of a dietary supplement. Surprisingly, the occurrence of various ailments related to the consumption of supplements was declared by as many as 64% of the respondents, and despite this, as many as 78% of the respondents declared exceeding the recommended daily dose of supplements (23). Our research shows a greater awareness of the dosage and use of dietary supplements by students of medical universities. This is evidenced by the fact that only 3.5% of women and 4.8% of men reported side effects after using dietary supplements. The knowledge is also evidenced by the fact that 75.2% of students in the first three years and 76.0% of students in further years correctly indicated the definition of a dietary supplement.

As part of this work, we have shown that only 1.5% of FM students, 1.3% of FP, and 2.7% FHS students pay attention to the packaging. The

packaging has not only a marketing function but also an informational, protective, logistic, ecological, and economic function (24). Research conducted in the United States carefully evaluates the packaging of vitamin preparations for children in terms of marketing, which may indicate that this population attaches more importance to this parameter. The authors analyzed 52 packages of supplements. Among the marketing activities aimed at children, the most frequently indicated were: the use of words related to taste and shape (88.5% of packaging), while among the activities addressed to parents: description of body functions or structures (82.7%) and expressions praising the lack of artificial flavors, colors, preservatives and sweeteners (also 82.7%) (25).

It was shown that 1.9% of FP students sell dietary supplements under MLM (Multi-level Marketing). Opinions about this method of selling differ. For example, Cardenas and Fuchs-Tarlovsky assessed that this method of sale by doctors and nutritionists may be subject to a potential conflict of interest (26).

A study by Spiólek et al. (2011) showed that students most often use dietary supplements to improve the condition of hair, nails, and skin (as many as 75% of respondents), the second reason is convalescence after illness and preventive use of dietary supplements (44.5%). In the above study, students of 4 fields of study (biotechnology, pharmacy, medical analytics, and cosmetology) also indicated what factors they take into account when choosing a vitamin and mineral preparation. Students primarily pay attention to the composition (89%, 96%, 84% and 90% of students of the above-mentioned fields, respectively), then to the price (51%, 31%, 37% and 46%) and the doctor's recommendation (19%, 29%, 24% and 14%). Marketing considerations are taken into account by a small percentage of respondents: more students pay attention to advertising (6%, 2%, 10% and 28%) than to packaging (2%, 2%, 0% and 0%) (27). The obtained results, presenting the main factors, are in line with the results obtained in our study, according to which advertising is important only for 2.5% of FP students and 6.8% of FHS students, while 0.0% of FM students.

A favorable tendency, which we have shown in our research, is the fact that students of medical university obtain knowledge about dietary supplements mainly from the Internet and studies - in the case of FP students, studies were indicated by 80.6% of the respondents, and the Internet by 65.6%. The Internet was also indicated by students in the study by Spiólek et al. (2011) as the main source of knowledge: it was

53%, 50%, 51%, and 66% of students of the above-mentioned fields, respectively (27).

Advertising is defined as a form of presentation and promotion of ideas, services, or goods, using e.g. press, transmission media, networks, media, and visual media, including through billboards and posters (7). Students are overwhelmed, for example, with the number of advertisements in advertising blocks, repeated advertisements for the same products, or being misled.

Lobb (2012) published a scientific fact-check that dealt with marketing claims made by mango-steen juice dietary supplements. He showed that many terms are imprecise and that the conclusions are too hasty. For example, marketing terms included the statement that the use of juice for 8 weeks lowers C-reactive protein levels. However, this study was conducted only on 40 participants, so it was not possible to detect side effects, e.g. with a frequency of 1% (28).

We showed that both among students in their first years and subsequent years, the average monthly expenditure on dietary supplements is in the range of PLN 20-40 (35.5% and 29.6% respectively). According to the data of the Central Statistical Office, in 2019 Poles spent an average of 25.1% of their income on food and beverages, and the average income per 1 person in the adult population in 2019 was PLN 1 819 (29).

Our research shows that students most often chose dietary supplements that contained vitamin D and / or K and dietary supplements with omega 3 and / or omega 6 and / or omega 9. Earlier literature data indicated that the most popular dietary supplements are preparations with magnesium (7.56%) (6). According to our survey results, 8.33% of students use magnesium supplements. The current epidemiological situation may cause such a large increase in interest in dietary supplements supporting immunity.

Our research also has some limitations. This study was conducted with MUB students - it is a group of people who care about their health, including a properly balanced diet and physical activity. The results of the survey regarding opinions on the marketing of dietary supplements may be different in the case of other populations, for example among students of technical faculties. Further research should also focus on a different selection of the study group: an equal gender distribution and an equal percentage of students from different faculties. The results of the above study also suggest that the composition and labeling of dietary supplements should be more supervised and controlled to ensure the safety and

effectiveness of supplementing the diet with dietary supplements.

To conclude, an issue that needs to be developed is a survey among medical university students and health care workers in Poland, regarding their knowledge and opinions about dietary supplements and marketing. These opinions should be taken into account when developing marketing strategies so that advertisements for dietary supplements are not misleading and discouraging, and provide factually correct information, in a condensed form and present dietary supplements with a confirmed composition, which will increase the confidence of patients and health care professionals.

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CONFLICT OF INTEREST

The authors report no conflicts of interest.

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Publikacja H8

Article

Is the Magnesium Content in Food Supplements Consistent with the Manufacturers' Declarations?

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Abstract: Food supplements (FS) are gaining more and more popularity because they are a quick way to compensate for deficiencies in the diet. Due to their affordable price and easy-to-take form, they are eaten by all age groups and by healthy and sick people. There are many categories of this type of preparations on the market, and FS with magnesium (Mg) are some of the most commonly used. Therefore, the aim of the study was to determine the Mg content in FS and to compare the estimated value with that declared by the manufacturer. The study included 116 FS containing Mg. In order to determine the Mg content, the atomic absorption spectrometry (AAS) method was used. The tested FS were divided in terms of the declared content, pharmaceutical form, chemical form of Mg, composition complexity, and price. It was shown that in the case of 58.7% of the samples, the Mg content was different than the permissible tolerance limits set by the Polish chief sanitary inspectorate, which range from −20% to +45%. It has been estimated that as a result of the differences in the content, the patient may take up to 304% more Mg per day or 98% less than it is stated in the declaration. The above results indicate that the quality and safety of FS should be more closely monitored.

Keywords: magnesium; pharmacy; food supplements; drugstore



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1. Introduction

Food supplements (FS) are preparations that are intended to supplement the diet with deficient substances. They contain minerals, vitamins, and other substances that can cause a specific physiological effect, such as fatty acids, amino acids, or probiotics. They come in various pharmaceutical forms, including tablets, capsules, powders, and ampoules. They do not contain medicinal substances, so they cannot be used to treat disease entities [1].

Minerals and vitamins that may be used in FS in Poland are listed in the Regulation of the Minister of Health of 17 September 2018. The minimum amount of vitamins and minerals in a daily portion in FS should not be less than 15% of the reference consumption values. However, maximum acceptable levels are established on the basis of the upper safe levels of consumption, the amounts provided in the diet, and the recommended intake for the population. This value must be safe for the health and life of the consumer [2].

FS are very common, and their popularity and market share are steadily increasing. Data from 2019 indicate that more than half of adults, both in Europe and the US, use FS [3]. According to research by Li et al. (2010), these are more often women than men (38.6% vs. 28.5%), as well as people over 50 (57.4%) and with higher education [4]. This is a consequence of the increased demand for nutrients in these groups. Despite the fact that FS cannot replace a balanced diet, their intake allows them to supplement existing deficiencies. In the elderly, it is particularly important because physiological changes in the body with age and long-term use of many medications make seniors most exposed to nutrient deficiencies.

Our previous research has shown that the use of FS is very widespread. Supplements containing magnesium (Mg) are used by approximately 8% of medical university students [5]. Other data from Poland indicate that FS with Mg account for 7.56% of the market. Preparations with this ingredient are very popular in Poland—about 25.0% of respondents use them. Among the inhabitants of Spain, 13.4% of men take them and among the inhabitants of Germany 18.3% of men and 20.4% of women [6].

The legal regulations governing the FS market in Poland are both national and European requirements. An important legal act is the Food and Nutrition Safety Act of 25 August 2006 [1], Regulation of the Minister of Health of 18 May 2010 amending the regulation on the composition and labeling of FS [7] and the Regulation of the Minister of Health of 17 September 2018, on the composition and labeling of FS [2]. The Regulation of the Minister of Health of 17 September 2018 [2] lists vitamins and minerals and their chemical forms that may be present in supplements. For substances authorized to appear in supplements, which were listed in the above regulation [2], further purity criteria are defined. Dyes and additives should meet the purity requirements specified in Commission Regulation (EU) No 231/2012 of 9 March 2012 [8]. Despite the above-mentioned legal acts being in force, the registration procedures and placing the FS on the market are very simple and only require presentation documentation and packaging design to the chief sanitary inspector. No quality or safety tests are required, which creates a risk that there are low-quality preparations on the market that differ in terms of their composition from the manufacturers' declarations.

Mg is necessary, *inter alia*, to maintain normal cell function, muscle contraction, including the heart muscle, and conditions nervous excitability [9,10]. Mg deficiency has also been shown to contribute to the development of oxidative stress in obese people [11], disturbances in mineral homeostasis such as Mg may interfere with cancer progression [12], and Mg supplementation may play a beneficial role in controlling asthma by acting as an anti-inflammatory and bronchodilator [13].

The causes of Mg deficiency include: reduced gastrointestinal absorption, loss of Mg from gastrointestinal tract, increased renal loss, excessive sweating, increased requirements (for example in pregnancy), or older age, which disrupts many processes [14].

The above factors prompted us to evaluate the Mg content in FS available on the Polish market, coming from local producers, but also producers known in various European countries. To our knowledge, this is the first study that covers such data as a variety of chemical forms, pharmaceutical forms or preparations at different prices.

2. Materials and Methods

2.1. Materials

Samples of dietary supplements were selected on the basis of previously conducted surveys [5] and on the basis of popularity in the largest chain pharmacies in the country.

Inclusion criteria included: popularity among patients, availability category 'FS', preparations within the expiry date.

The following exclusion criteria were adopted: occasional sales, over-the-counter 'OTC' availability category.

The study included 116 FS purchased in stationary pharmacies as well as online. In order to assess the quality of FS in the best possible way, preparations were selected for the research, which differed in terms of composition, pharmaceutical form, price and, manufacturer. Detailed characteristics of the studied FS are presented in Table S1 in Supplementary Materials.

FS were taken from three different blisters or as three subsamples, analyzed in triplicate (statistically insignificant differences between the determinations) were harvested and tested in 2020–2021.

2.2. Sample Preparation

FS were ground in a vibrating grinder (Testchem, Poland) and weighed into Teflon mineralization vessels of about 0.3 g with an accuracy of 1 mg (exact weights were recorded). Then 4 mL of spectrally pure concentrated (69%) nitric acid (Tracepur, Merck, Darmstadt, Germany) were added. The microwave mineralization process was carried out in a closed system (Berghof, Speedwave, Eningen, Germany), according to the following program:

- Step 1: 170 °C, 10 min, 20 atm., 80% of microwave power;
- Step 2: 190 °C, 10 min, 30 atm., 90% of microwave power;
- Step 3: 210 °C, 10 min, 40 atm., 90% of microwave power;
- Step 4: 50 °C, 18 min, 40 atm., 0% of microwave power.

The obtained mineralizates were quantitatively transferred to polypropylene vessels with deionized water.

2.3. Determination of Mg Content

Mg content was determined by atomic absorption spectrometry (AAS), acetylene-air flame technique with Zeeman background correction. The determination was carried out using the Z-2000 instrument (Hitachi, Tokyo, Japan). Before the analysis, all of the analyzed samples were diluted, depending on the declared content of the tested element. Lanthanum chloride (1% LaCl₃, Sigma-Aldrich, Merck, Darmstadt, Germany) was used as the sequestering agent. The assay was performed at a wavelength of 285.2 nm and 7.5 mA current lamps. The concentration was read from the curve prepared using a 1 mg/mL Mg standard solution for AAS (Merck, Germany). The limit of detection (LOD) and limit of quantification (LOQ) were 0.26 mg/kg and 0.78 mg/kg, respectively.

The conducted research did not require the approval of the Bioethics Committee of the Medical University of Białystok.

2.4. Validation of Method

In order to control the accuracy of the analyses, a certified reference material was used (Simulated Diet D, LIVSMEDELS VERKET, National Food Administration, Uppsala, Sweden). The determination was performed before the analysis and after each 10 determinations. All values were within the certified value range (2740–3100 mg/kg). The accuracy (% of error) was 0.67%, and the coefficient of variation $V = 1.57\%$.

2.5. Comparison of Results with the Standards Adopted by the Chief Sanitary Inspectorate in Poland

In accordance with the guidelines adopted by the European Commission in 2012 on establishing tolerance limits for minerals contained on labels, the obtained values were compared with the guidelines adopted by the Commission, amounting to −20 to +45% for FS-containing minerals [15,16].

2.6. Statistical Analyses

Statistica software (Tibco, Palo-Alto, CA, USA) was used for calculations and statistical analyzes. The results are presented as mean (Av.) with standard deviation (SD), minimum (Min), maximum (Max), as well as median (Med.), and lower quartile (Q1), upper quartile (Q3), interquartile range (IQR).

3. Results

The results of the analyses are presented in Tables 1–5. The following classification criteria were used: declared content, pharmaceutical form, chemical form of Mg, amount of minerals (only Mg or multi-component preparations), and price.

Table 1. Magnesium content (mg/portion) in food supplements depending on the declared magnesium content.

Declared Content	n	Mg Content (mg/Portion)					
		Av. \pm SD	Min–Max	Med.	Q1	Q3	IQR
Less than 100 mg	49	49.7 \pm 38.0	1.5–202.0	40.7	23.7	73.4	49.7
100–200 mg	45	144.9 \pm 109.5	7.4–469.6	115.7	66.3	207.2	141.0
Above 200 mg	22	387.0 \pm 200.2	39.1–795.7	348.7	249.2	479.2	230.0

Av.—average, IQR—interquartile range, Max—maximum value, Med.—median, Min—minimum value, Q1—lower quartile, Q3—upper quartile, SD—standard deviation.

Table 2. Magnesium content (mg/portion) in food supplements depending on the pharmaceutical form.

Form	n	Mg Content (mg/Portion)					
		Av. \pm SD	Min–Max	Med.	Q1	Q3	IQR
Capsules	13	103.8 \pm 110.0	1.5–298.5	69.2	22.8	193.0	170.2
Coated tablets	11	68.5 \pm 60.4	19.0–202.0	48.0	23.7	77.5	53.8
Dragees	2	78.3 \pm 22.7	62.2–94.3	78.3	62.2	94.3	32.1
Effervescent tablets	24	231.2 \pm 196.0	4.9–696.9	168.2	78.6	364.2	285.6
Granulates	1	233.1 \pm 0.0	-	-	-	-	-
Jelly beans	1	27.5 \pm 0.0	-	-	-	-	-
Liquids	7	198.4 \pm 120.6	34.0–360.1	219.7	75.4	317.6	242.2
Powders	12	264.2 \pm 247.2	22.1–795.7	189.1	81.2	367.4	286.2
Tablets	45	106.8 \pm 133.6	5.8–696.5	60.2	31.4	120.8	89.4

Av.—average, IQR—interquartile range, Max—maximum value, Med.—median, Min—minimum value, Q1—lower quartile, Q3—upper quartile, SD—standard deviation.

Table 3. Magnesium content (mg/portion) in food supplements depending on the chemical form.

Chemical Form	n	Mg Content (mg/Portion)					
		Av. \pm SD	Min–Max	Med.	Q1	Q3	IQR
Magnesium bisglycinate	6	161.4 \pm 103.1	28.5–317.6	154.3	93.8	219.7	126.0
Magnesium carbonate	34	132.2 \pm 164.2	5.8–696.9	73.9	40.7	137.8	97.1
Magnesium citrate	35	168.4 \pm 201.1	1.5–795.7	79.1	31.5	232.0	200.6
Magnesium glycerophosphate	1	78.8 \pm 0.0	-	-	-	-	-
Magnesium hydroxide	2	215.6 \pm 263.8	29.1–402.1	215.6	29.1	402.1	373.0
Magnesium lactate	11	45.7 \pm 39.4	1.8–129.3	35.4	7.4	77.9	70.5
Magnesium oxide	8	207.6 \pm 155.5	18.8–449.4	225.6	52.9	317.9	265.1
Several chemical forms	19	181.2 \pm 148.1	22.8–479.2	145.4	61.2	267.6	206.4

Av.—average, IQR—interquartile range, Max—maximum value, Med.—median, Min—minimum value, Q1—lower quartile, Q3—upper quartile, SD—standard deviation.

Table 4. Magnesium content (mg/portion) in food supplements depending on the amount of minerals.

Amount of Minerals	n	Mg Content (mg/Portion)					
		Av. \pm SD	Min–Max	Med.	Q1	Q3	IQR
Only magnesium (or vitamin B6)	75	164.8 \pm 183.6	1.5–795.7	93.8	34.0	249.2	215.3
Multicomponent preparations	41	124.7 \pm 125.6	4.8–469.6	76.4	38.5	188.8	150.2

Av.—average, IQR—interquartile range, Max—maximum value, Med.—median, Min—minimum value, Q1—lower quartile, Q3—upper quartile, SD—standard deviation.

Table 5. Magnesium content in food supplements depending on the price.

Price (PLN)	n	Mg Content (mg/Portion)					
		Av. \pm SD	Min–Max	Med.	Q1	Q3	IQR
<10	41	192.1 \pm 191.1	13.3–696.9	108.3	35.4	317.6	282.1
10–20	57	112.0 \pm 138.8	1.5–795.7	74.4	30.5	129.3	98.7
>20	18	178.4 \pm 164.2	22.8–649.8	113.1	71.5	267.6	196.1

Av.—average, IQR—interquartile range, Max—maximum value, Med.—median, Min—minimum value, PLN—currency in force in Poland, Q1—lower quartile, Q3—upper quartile, SD—standard deviation.

In the case of preparations with a declared content below 100 mg of Mg per portion, the highest determined content was 202.0 mg, and the lowest was only 1.5 mg (Table 1).

The second criterion of the division was the criterion of the pharmaceutical form. The formulation with the highest reported content (795.7 mg/portion) was available as a powder to be dissolved in water. The lowest marked values were for supplements available in the form of capsules (1.5 mg/portion), effervescent tablets (4.9 mg/portion), and tablets (5.8 mg/portion).

Most of the studied FS contained Mg in the form of Mg citrate ($n = 35$) and Mg carbonate ($n = 34$), while the least contained in the form of hydroxide ($n = 2$) and glycerophosphate ($n = 1$). Both the preparation with the lowest determined content of Mg (1.5 mg/portion) and the preparation with the highest determined content (795.7 mg/portion) contained Mg citrate (Table 3).

Out of 116 FS tested, 75 contained only Mg among the minerals. This category includes both the preparation with the lowest determined value (1.5 mg) and the preparation with the highest determined mg content (795.7 mg) (Table 4).

Interestingly, in preparations with a lower price (below PLN 10), the highest mean Mg content was recorded at the level of 192.1 ± 191.1 (Table 5).

The chief sanitary inspectorate, responsible for the quality of FS, allows the deviation of minerals from -20% to $+45\%$. Figure 1 shows the variation in individual samples. It was shown that 58.7% of FS were outside the acceptable range (Figure 1a,b).

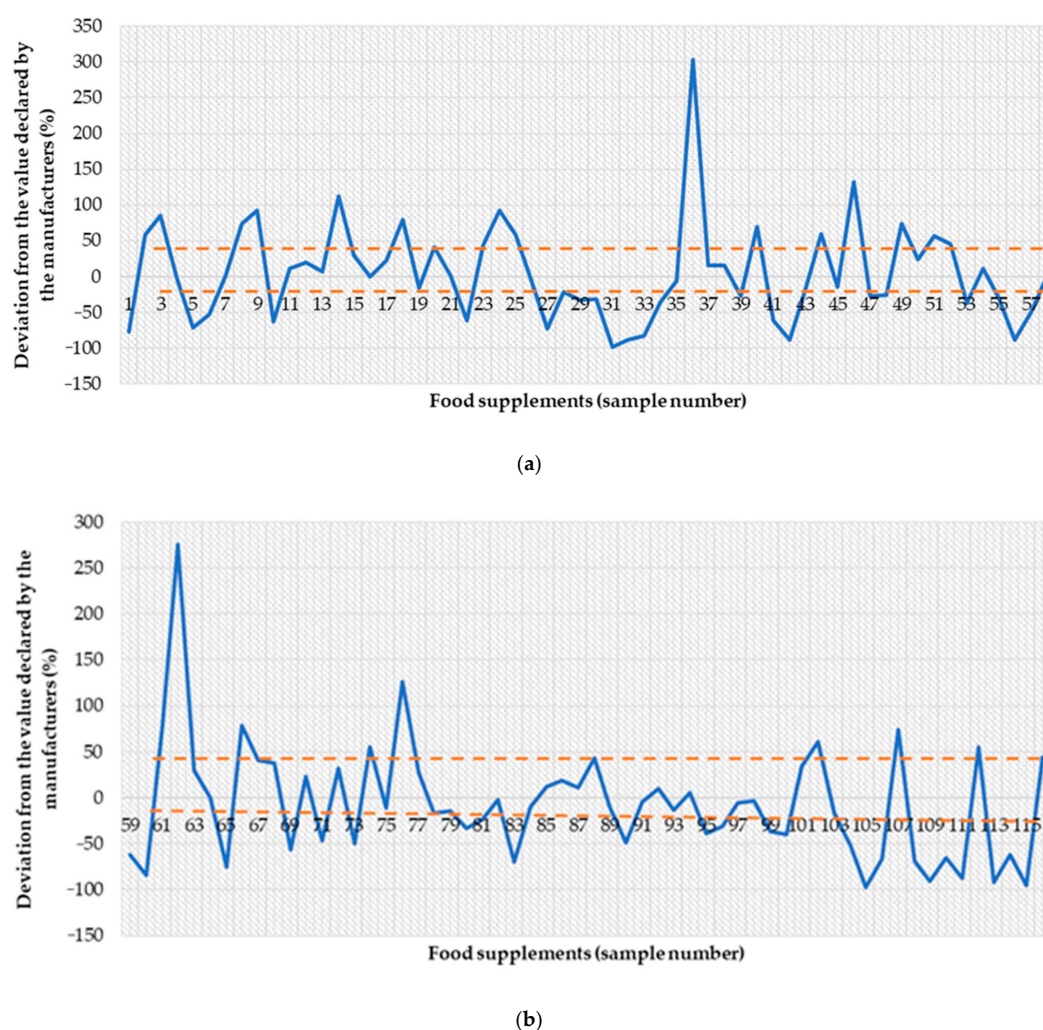


Figure 1. The discrepancy between the declared magnesium content and that determined in dietary supplements (a) samples of food supplements from 1 to 58, (b) samples of food supplements from 59 to 116.

At a further stage, we also assessed by how many percent the expected value by consumers would differ from the actual value consumed, in accordance with the manufacturer's recommendation, because the tested FS can be taken in amounts greater than just one portion per day. It has been shown that for 54.1% of FS, consumers will consume a lower amount of Mg. For example, for 3.4% it will be 90–100% less than the expected value. Worryingly, in the case of one of the studied FS, consumers, using one portion of the FS each day, will consume as much as 300% more Mg than indicated on the packaging (Figure 2).

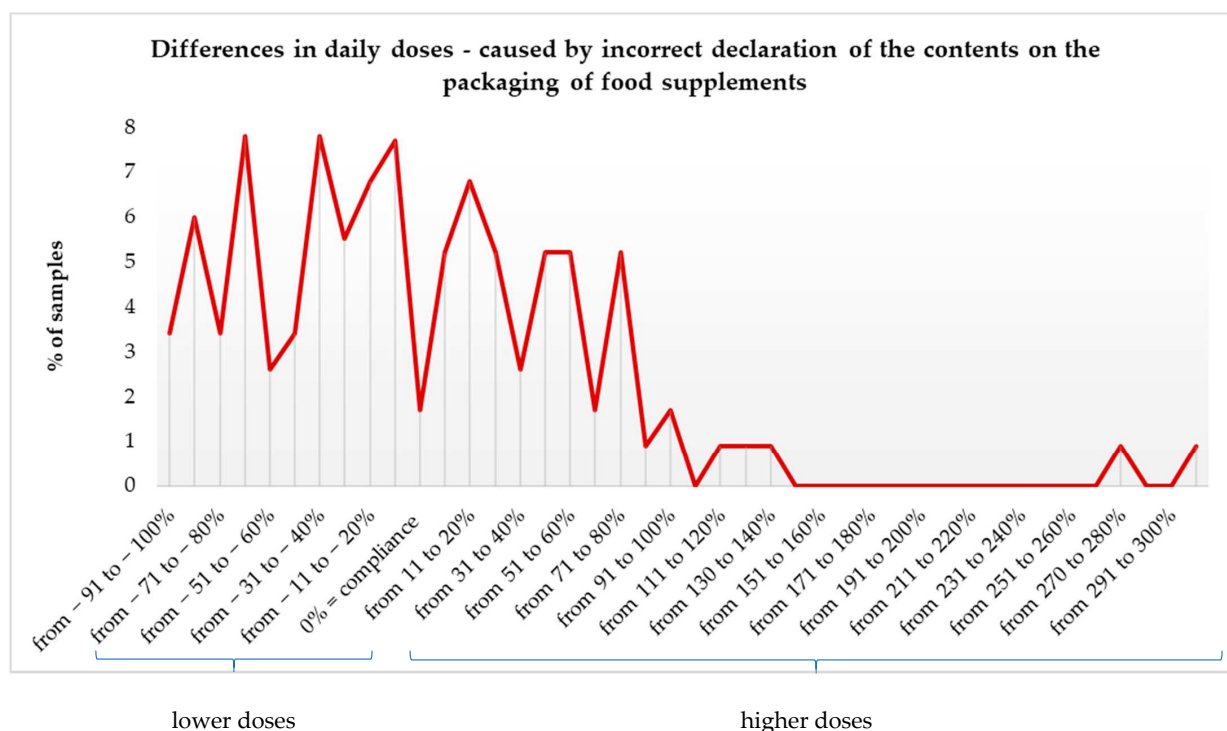


Figure 2. The percentage of food supplements and the difference in the actually taken magnesium dose, resulting from an incorrect declaration of the content.

There were no statistically significant differences ($p > 0.05$) between the previously discussed factors (declaration of Mg content, pharmaceutical form, chemical form, composition, price) and the percentage of samples within the norm, below and above the norm (Table 6).

Table 6. Relationship between factors and percentage of food supplements with normal, below and above normal magnesium levels ($p > 0.05$).

Criterion	Subgroups	<i>n</i>	Below Standard <i>n</i> = 46 (%)	Norm <i>n</i> = 48 (%)	Above Normal <i>n</i> = 22 (%)
Declared content	Less than 100 mg	49	19 (16.4)	24 (20.7)	6 (5.2)
	100–200 mg	45	20 (17.2)	15 (12.9)	10 (8.6)
	Above 200 mg	22	7 (6.0)	9 (7.6)	6 (5.2)
Form	Capsules	13	9 (7.8)	3 (2.6)	1 (0.9)
	Coated tablets	11	5 (4.3)	5 (4.3)	1 (0.9)
	Dragees	2	0 (0.0)	2 (1.7)	0 (0.0)
	Effervescent tablets	24	5 (4.3)	12 (10.3)	7 (6.0)
	Granulates	1	0 (0.0)	0 (0.0)	1 (0.9)
	Jelly beans	1	0 (0.0)	1 (0.9)	0 (0.0)
	Liquids	7	3 (2.6)	3 (2.6)	1 (0.9)
	Powders	12	3 (2.6)	4 (3.4)	5 (4.3)
	Tablets	45	21 (18.1)	18 (15.5)	6 (5.2)

Table 6. Cont.

Criterion	Subgroups	<i>n</i>	Below Standard <i>n</i> = 46 (%)	Norm <i>n</i> = 48 (%)	Above Normal <i>n</i> = 22 (%)
Chemical form	Magnesium bisglycinate	6	2 (1.7)	3 (2.6)	1 (0.9)
	Magnesium carbonate	34	11 (9.5)	15 (12.9)	8 (6.9)
	Magnesium citrate	35	16 (13.8)	12 (10.3)	7 (6.0)
	Magnesium glycerophosphate	1	0 (0.0)	1 (0.9)	0 (0.0)
	Magnesium hydroxide	2	1 (0.9)	0 (0.0)	1 (0.9)
	Magnesium lactate	11	6 (5.2)	5 (4.3)	0 (0.0)
	Magnesium oxide	8	3 (2.6)	4 (3.4)	1 (0.9)
	Several chemical forms	19	7 (6.0)	8 (6.9)	4 (3.4)
Amount of minerals	Only magnesium (or vitamin B6)	75	35 (30.2)	25 (21.6))	15 (12.9
	Multicomponent preparations	41	11 (9.5)	23 (19.8)	7 (6.0)
Price (PLN)	<10	41	16 (13.8)	17 (14.7)	8 (6.9)
	10–20	57	25 (21.6)	22 (18.9)	10 (8.6)
	>20	18	5 (4.3)	9 (7.8)	4 (3.4)

4. Discussion

FS are foodstuffs taken by patients and consumers to supplement existing deficiencies. Their use is not intended to treat or prevent diseases in humans, unlike drugs, and are not required to be subject to detailed qualitative and quantitative research prior to sale, unlike medicinal products. Moreover, their side effects are not monitored rigorously. This generates the need to test their quality. Our research covered more than 100 FS, which may reflect the assortment of the largest pharmacy chains.

As part of the study conducted by the SW RESEARCH agency (2017), a survey was conducted among 807 adults. It was estimated that 72.4% of Poles use FS, and about half of them systematically, i.e., 48%. Worrying is the fact that only 17% consult a doctor or pharmacist before starting supplementation. The most common reasons for taking these preparations were the desire to strengthen the body (55.4%), increase resistance to infections (44.3%), and supplement the daily diet with the missing ingredients (40.7%). Alarming, 6% of people argued taking supplements is the current fashion. The respondents declared that during the purchase they were guided by the composition (41.7%), price (38%), their own experience (36.6%), the recommendation of a doctor or pharmacist (34.5%), opinions of other people (25.9%), scientific certificates (16.9%) and other factors (27.7%). Satisfactory is the fact that the most frequent place of purchase was the pharmacy (65%). According to 54.9% of people, taking supplements brought them noticeable benefits, while 41.6% did not notice an effect on their health, while 3.4% were dissatisfied with the effects. In this study, more frequent use of supplements by women (51.7%) than men (48.3%) was observed, as well as among people with higher education (45.5%) [17].

Mg, next to potassium, is the most important intracellular cation. It activates over 300 enzymes. It participates, among others, in neuromuscular conduction, regulation of the body's mineral homeostasis, regulation of blood pressure, insulin metabolism, and muscle contractility. It is a macronutrient necessary for proper functioning, therefore it should be supplied with a balanced diet. A number of factors, including the consumption of highly processed food, contribute to its reduced amount in the diet [18].

It is disturbing that if one of the tested FS is consumed, the patient will be take in 300% more Mg every day than it is stated in the declaration on the packaging. Taking too much of a dose may have side effects. There is no evidence that food-derived Mg can have a negative effect on the body, while in the case of excessive consumption of Mg from various types of supplements or medicinal products, cases of harmful effects have been reported. Since Mg salts are laxative when used in large amounts, osmotic diarrhea may occur. Symptoms also include difficulty breathing, sleep disturbances, changes in heartbeat, muscle weakness and confusion. In extreme cases, when it is accompanied by impaired renal function, serious neurological symptoms may occur, such as, among others, increased

axonal excitability threshold, paralysis of the striated and cardiological muscles, including inhibition of heart contractions or prolongation of the QT interval [19]. Serious side effects, including death, were found after children took 2400 mg of Mg, which is three servings of the supplement with the highest content determined in this study [20]. The maximum amount of Mg allowed in FS is 400 mg/day [21], while our study showed almost twice as much Mg in one of the preparations.

The subject of comparing the declared and determined Mg content has so far been rarely discussed in scientific publications. Literature data indicate that the interest of researchers in FS containing Mg is also focused on assessing the safety of their use, due to the presence of potential contaminants [22,23].

It seems necessary to conduct patient education and large-scale campaigns. A 2014 study showed that every fourth Pole was unable to correctly define the definition of a FS. Almost half of the people, as many as 41%, claimed that these products have healing properties, while 31% of people assessed that they were synonymous with vitamins, and 8% that they were synonymous with minerals. Moreover, half of the respondents (50%) believed that they were subject to the same control as drugs [24].

The presence of a large amount of FS on the market makes their control very difficult. As a result of the easy registration process, more and more of them appear on the market. Data from Poland show that from 2007 to 2017, over 29,000 were entered into the register of products in the FS category. In 2016, the chief sanitary inspector received about 600 notifications about the introduction of a new preparation on the market every month [6]. This indicates the need to introduce greater restrictions, preventing the placing on the market of preparation of inappropriate quality.

Ensuring an adequate level of Mg in the body is essential for its proper functioning. The benefits of using it have been reported even in diseases of various pathogenesis, e.g., ulceration [25]. As a result of taking FS of inadequate quality, containing several times the lower doses of Mg than declared by the manufacturer, supplementing the deficiencies may be ineffective, which may result in the lack of the effects expected by patients.

The absorption of Mg is greater from food than from supplements [26–29]. Therefore it is necessary to properly balance the diet so that taking these preparations will not be necessary. However, when it is impossible or when there is an increased demand for this element as a result of other factors, supplementation with high-quality preparations should be used [30,31]. A diet deficient in terms of Mg is quite common. For example, a 2009 study assessed eating habits among people living in Russia, the Czech Republic and Poland. Consumption in line with the recommendations of the standards was shown only in about 65% of the respondents. The highest consumption of this element was among Poles, who consumed 286 mg. Czechs supplied 278 mg and Russians only 97 mg [32].

Such a large discrepancy between the declared values and those actually marked is very surprising. It may result from the improper production process of FS, lack of final product control, and inadequate labeling of the supplement. FS are sold in grocery stores, drugstores, herbal, and medical stores and pharmacies. It seems necessary that FS sold in pharmacies should be subject to greater control, which will improve their quality and increase consumer confidence in this category of food products, sold in a form analogous to drugs.

The limitations of this study are as follows: due to the heterogeneity of FS quality, other batches may have a different Mg content, the study describes the most popular preparations on the market, although the quality of FS produced by niche producers may be different. The limitation is also the large market share of preparations in one pharmaceutical and chemical form. Further research should be based on the assessment of the bioavailability of various Mg compounds and the actual concentration obtained in the blood of patients after FS ingestion.

5. Conclusions

The assessment of the quality of food containing magnesium showed that the declared and actual values in most dietary supplements differed. Only in two samples of the supplements were they identical. Only 41.3% of the tested samples were within the acceptable range of deviations for minerals, in line with the recommendations of the chief sanitary inspectorate. During the intake of food supplements covered by this study by patients, as a result of differences between the declared value and the measured value, the amount of the consumed element may change in the amount from a maximum of 98% less to 304% more than the declared value. Food supplements should be routinely monitored to improve their quality.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/nu13103416/s1>, Table S1: Characteristics of the studied dietary supplements.

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Publikacja H9

Article

Quality of Calcium Food Supplements: Evaluation Compared to Manufacturers' Declarations

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Abstract: Calcium (Ca) is a macronutrient necessary for the proper functioning of an organism. In the case of insufficient consumption with diet, its deficiencies can be supplemented with food supplements (FS). These supplements are used, for example, as an auxiliary in the prevention of osteoporosis, allergies, hair loss or nail brittleness. The purpose of the study was to assess the compliance of Ca content with the manufacturers' declaration. The material consisted of 108 FS. Ca content was determined by atomic absorption spectrometry (AAS). It was shown that 1.9% of the samples were characterized by a Ca content that was too low in comparison to the manufacturer's declaration, while a content that was relatively too high was found in 54.6% of FS. The quality of FS should be monitored to ensure patient safety.

Keywords: food supplements; calcium; osteoporosis; skin; hair and nails; nutricosmetics; allergy



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1. Introduction

Food supplements (FS) are foods that are intended to supplement a normal diet. FS are a concentrated source of nutrients or other substances that have a nutritional or physiological effect. Supplements can contain one ingredient or more. They are available for sale in a form that allows patients to precisely dose (e.g., tablets, capsules, sachets) [1].

The use of dietary supplements is very widespread. According to the report "Poles and dietary supplements", over 67% of Poles declare the use of supplementation, and nearly 76% emphasize that they use it regularly. The most frequently taken preparations included vitamin and mineral supplements (81.1%), supplements to improve the appearance of skin, hair and nails (33.2%) and supplements to strengthen joints and bones (31.4%). These FS categories may include, inter alia, calcium (Ca). Worryingly, over 45% of consumers do not consult any specialist on purchasing supplements [2,3].

In recent years, research in different countries on FS has focused mainly on quality assessment, including the determination of the content of impurities such as mercury [4,5], arsenic [6], cyanotoxins [7], polycyclic aromatic hydrocarbons [8], as well as drug components that should not be present in FS, such as potency substances [9] and pharmaceutical adulterants, such as anxiolytics, antidepressants, anorexics, stimulants and laxatives [10]. A significant problem that is not addressed in scientific research is incorrect content of the declared ingredients in FS.

Despite the widespread use of FS, it should be emphasized that their quality is not sufficiently controlled. A comprehensive assessment of the quality of the FS market in Poland was carried out by the Supreme Chamber of Control in 2017–2020. Its aim was to assess the safety of placing FS on the market and trading them. The summary report shows that due to the lack of an appropriate monitoring system, consumers may have been exposed to inadequate quality preparations for many years. This is primarily related to the intensive development of the supplement industry and the insufficient capacities of the regulatory authorities. From 2017 to 2020, only in Poland itself, over 62,000 notifications of

the first marketing of FS were submitted. With regard to approximately 56,000 preparations, the verification process was not undertaken at all. In some cases, the verification process took several years, and the analyzed preparations could still be available for sale [11]. An important aspect is also the discrepancy between the declared and marked content. This research problem is not addressed sufficiently in publications.

Ca is a mineral that is essential for the functioning of the human organism. In an adult, it constitutes about 2% of body weight, which is on average about 1200 g [12]. Ca plays a major role in the conduction of nerve stimuli, hormonal regulation, muscle contractility and the activation of certain enzymes [13].

According to the legislation, 15 minerals, including Ca, can be contained in FS. Ca may be present in the following combinations: Ca carbonate, Ca chloride, Ca gluconate, Ca glycerophosphate, Ca hydroxide, Ca lactate, Ca oxide, Ca salts of citric acid and Ca salts of orthophosphoric acid [1]. Pursuant to the resolution of the Committee for Dietary Supplements, operating at the Sanitary and Epidemiological Council, the maximum content of Ca in the recommended daily dose of FS intended for consumption by adults is 1500 mg [14]. This is based on the European guidelines, which indicate the Maximum Supplement Level (MSL) [15]. The permissible deviation of the content of the mineral component is from −20% to 45% declared value [16,17].

Therefore, the objective of this study was to assess the Ca content in various FS available on the market in Poland in relation to the manufacturers' declarations regarding the Ca content.

2. Results

The results regarding the Ca content in selected groups of FS, taking into account several classification criteria, are presented in Tables 1–8. The results were presented as one serving or one dose (1 tablet, 1 capsule etc.). The differences in the doses taken during the day were converted into daily doses (taking into account the number of doses recommended for consumption during the day).

Table 1. Calcium content in food supplements, taking into account the pharmaceutical form.

Pharmaceutical Form (Sign)	n	Ca Content (mg/dose)					
		Δ (%)					
		Av. \pm SD	Min–Max	Med	Q1	Q3	IQR
Capsules (1)	6	243.6 \pm 160.0	75.8–540.5	217.0	152.6	258.9	106.3
		70.5 \pm 77.5	from −9.0 to 209.9	63.5	9.0	86.1	77.1
Effervescent tablets (2)	44	278.7 \pm 150.1	58.3–595.1	227.0	162.2	365.7	203.5
		1992.9 \pm 12,905.7	from −34.7 to 85,653.2	41.8	13.4	72.7	59.3
Powders (3)	6	588.1 \pm 992.5	122.4–2610.0	177.3	141.5	300.3	158.9
		63.2 \pm 114.6	from −65.2 to 275.0	47.8	2.0	71.6	69.6
Tablets (4)	49	498.8 \pm 407.0	31.0–1661.3	383.1 * _{4/5}	195.7	690.3	494.7
		90.0 \pm 182.3	from −13.8 to 1295.5	63.1	28.8	99.0	70.2
Other form (5)	3	65.4 \pm 44.5	17.1–104.1	74.6	17.1	104.6	87.5
		77.2 \pm 8.3	from 70.7 to 86.5	74.3	70.7	86.5	15.8

Av.—average, IQR—interquartile range, Max—maximum value, Med—median, Min—minimum value, Q1—lower quartile, Q3—upper quartile, SD—standard deviation, * $p < 0.05$, Δ (%)—the difference between the declared value (taken as 100%) and the determined value.

Table 2. Calcium content in food supplements, taking into account the price.

Price (PLN)	n	Ca Content (mg/dose)					
		Δ (%)					
		Av. \pm SD	Min–Max	Med	Q1	Q3	IQR
<10	44	278.4 \pm 152.5	58.3–595.1	234.0	161.6	365.7	204.1
		49.3 \pm 47.8	from –34.7 to 230.6	42.5	13.4	74.0	60.5
10–20	23	547.7 \pm 583.2	102.7–2610.0	383.1	225.7	647.7	422.0
		3853.0 \pm 17,833.7	from –4.9 to 85,653.2	65.8	28.3	128.0	99.7
>20	41	415.6 \pm 392.3	17.1–1389.2	221.2	156.8	534.8	378.0
		57.2 \pm 50.9	from –65.2 to 170.7	54.4	28.8	74.5	45.7

Av.—average, IQR—interquartile range, Max—maximum value, Med—median, Min—minimum value, Q1—lower quartile, Q3—upper quartile, SD—standard deviation, Δ (%)—the difference between the declared value (taken as 100%) and the determined value.

Table 3. Calcium content in the food supplements, taking into account the age group for which they are intended.

Age Group (Sign)	n	Ca Content (mg/dose)					
		Δ (%)					
		Av. \pm SD	Min–Max	Med	Q1	Q3	IQR
Children (1)	4	105.5 \pm 88.0	17.1–225.7	89.6 *	45.8	165.1	119.3
		57.2 \pm 50.9	from –65.2 to 170.7	1/2, 1/3 54.4	28.8	74.5	45.7
Children and adults (2)	20	338.7 \pm 230.0	102.7–1145.2	259.8	215.7	379.8	164.1
		3853.0 \pm 17,833.7	from –4.9 to 85,653.2	65.8	28.3	128.0	99.7
Adults (3)	84	413.0 \pm 415.6	31.0–2610.0	254.9	156.3	518.2	361.9
		49.3 \pm 47.8	from –34.7 to 230.6	42.5	13.4	74.0	60.5

Av.—average, IQR—interquartile range, Max—maximum value, Med—median, Min—minimum value, Q1—lower quartile, Q3—upper quartile, SD—standard deviation, * $p < 0.05$, Δ (%)—the difference between the declared value (taken as 100%) and the determined value.

Table 4. Calcium content in the food supplements, taking into account the country of the producer.

Country	n	Ca Content (mg/dose)					
		Δ (%)					
		Av. \pm SD	Min–Max	Med	Q1	Q3	IQR
Croatia	3	327.6 \pm 184.5	217.0–540.5	225.2	217.0	540.5	323.5
		34.7 \pm 17.2	from 22.6 to 54.4	27.2	22.6	54.4	17.2
Germany	6	527.0 \pm 539.7	154.6–1389.2	219.7	156.8	1021.6	864.7
		71.6 \pm 54.8	from –3.3 to 131.5	74.9	30.7	120.6	54.8
Japan	4	678.0 \pm 528.3	225.7–1294.1	596.0	237.7	1118.2	880.4
		106.8 \pm 45.3	from 54.2 to 158.8	107.1	71.3	142.2	70.9
Poland	81	378.4 \pm 391.8	31.0–2610.0	252.2	153.1	500.0	347.0
		1131.1 \pm 9509.9	from –34.7 to 85,653.2	51.7	18.8	82.1	63.3
United States	8	264.7 \pm 138.5	139.7–534.8	214.7	171.8	335.1	163.3
		25.7 \pm 45.8	from –65.2 to 72.6	33.3	4.9	60.7	55.9
Other	6	376.9 \pm 287.5	17.1–751.2	307.4	188.3	690.3	502.0
		58.0	from 25.8 to 72.6	63.8	50.2	71.6	21.4

Av.—average, IQR—interquartile range, Max—maximum value, Med—median, Min—minimum value, Q1—lower quartile, Q3—upper quartile, SD—standard deviation, Δ (%)—the difference between the declared value (taken as 100%) and the determined value.

Table 5. Calcium content in the food supplements, taking into account the chemical form of calcium.

Chemical Form	n	Ca Content (mg/dose)					
		Δ (%)					
		Av. \pm SD	Min–Max	Med	Q1	Q3	IQR
Calcium carbonate	72	455.6 \pm 428.6	31.0–2610.0	335.4	171.1	537.6	428.6
		1247.7 \pm 10,087.5	from –65.2 to 85,653.2	57	20.2	84.5	64.3
Calcium lactate	9	271.3 \pm 125.5	186.0–595.1	225.2	217.0	255.0	38.0
		54.4 \pm 68.7	from 3.3 to 230.6	27.2	22.7	42.9	20.3
Calcium salts of orthophosphoric acid	7	176.6 \pm 83.6	31.9–265.9	208.2	119.4	249.8	130.4
		41.2 \pm 35.9	from –13.8 to 99.0	36.5	18.2	64.2	45.9
Tricalcium phosphate	3	159.0 \pm 32.1	122.4–182.0	172.6	122.4	182.0	59.7
		32.5 \pm 26.7	from 2.0 to 51.7	43.9	2.0	51.7	49.7
Other forms	6	220.1 \pm 129.5	87.7–457.1	186.6	143.5	258.9	115.4
		65.2 \pm 20.0	from 35.0 to 95.0	67.0	54.4	72.6	18.2
Several chemical forms	7	440.3 \pm 425.8	152.6–1353.4	247.9	186.1	534.8	348.7
		89.9 \pm 74.3	from 9.0 to 209.9	67.1	23.5	170.7	147.1
No declaration	4	132.5 \pm 95.3	17.1–246.5	133.1	64.4	200.6	136.2
		350.0 \pm 631.1	from –3.3 to 1295.5	53.8	16.8	683.1	631.1

Av.—average, IQR—interquartile range, Max—maximum value, Med—median, Min—minimum value, Q1—lower quartile, Q3—upper quartile, SD—standard deviation, Δ (%)—the difference between the declared value (taken as 100%) and the determined value.

Table 6. Calcium content in the food supplements, taking into account the content declared by the manufacturer.

Declaration of the Content (Sign)	n	Ca Content (mg/dose)					
		Δ (%)					
		Av. \pm SD	Min–Max	Med	Q1	Q3	IQR
<100 (1)	16	96.9 \pm 55.7	17.1–247.9	95.3 **	66.5	115.5	49.1
		5496.1 \pm 21,377.5	from –9.0 to 85,653.2	1/2, 1/3 75.1	30.2	127.1	96.9
100–200 (2)	47	213.3 \pm 80.4	100.7–595.1	208.2 ***	167.5	249.8	82.3
		48.8 \pm 48.3	from –16.0 to 230.6	2/3 40.8	17.3	67.5	50.2
>200 (3)	45	673.7 \pm 451.7	141.5–2610.0	521.0	411.4	829.2	417.8
		64.4 \pm 59.0	from –65.2 to 275.0	63.3	25.8	77.2	51.4

Av.—average, IQR—interquartile range, Max—maximum value, Med—median, Min—minimum value, Q1—lower quartile, Q3—upper quartile, SD—standard deviation, ** $p < 0.01$, *** $p < 0.001$, Δ (%)—the difference between the declared value (taken as 100%) and the determined value.

Considering the pharmaceutical form, the tablets were characterized by the highest median Ca content (Table 1), this amount was significantly higher compared to the Ca content in the other pharmaceutical forms of FS (383.1 vs. 74.6 mg/dose).

Another criterion used was the price. Three subgroups were adopted: cheap preparations (price below PLN 10), medium-price preparations (PLN 10–20) and high-price preparations (over PLN 20), taking into account the average earnings in Poland. The highest median Ca content was shown in preparations with an average price, between PLN

10 and PLN 20. However, the median Ca content was not higher compared to the cheaper and more expensive preparations (Table 2).

Table 7. Calcium content in food supplements, taking into account the type of preparation.

Type of Food Supplement	n	Ca Content (mg/dose)					
		Δ (%)					
		Av. \pm SD	Min–Max	Med	Q1	Q3	IQR
Single	13	484.5 \pm 670.3	87.7–2610.0	255.0	220.8	337.1	116.3
		70.3 \pm 72.8	from 12.4 to 275.0	36.9	25.3	95.0	69.7
Complex	95	374.6 \pm 330.3	17.1–1661.3	249.8	154.6	506.4	351.8
		970.7 \pm 8781.7	from –65.2 to 85,653.2	54.4	21.7	77.2	55.5

Av.—average, IQR—interquartile range, Max—maximum value, Med—median, Min—minimum value, Q1—lower quartile, Q3—upper quartile, SD—standard deviation, Δ (%)—the difference between the declared value (taken as 100%) and the determined value.

Table 8. Relationship between criterions and percentage of food supplements with normal, below and above normal calcium levels.

Criterion	Groups	n (% of the Category)	Above Norm n (% of Subcategories)	In the Norm n (% of Subcategories)	Below Norm n (% of Subcategories)
Pharmaceutical form	Capsules	6 (5.56)	4 (66.67)	2 (33.33)	0 (0.00)
	Effervescent tablets	44 (40.74)	20 (45.45)	23 (52.28)	1 (2.27)
	Powders	6 (5.56)	3 (50.00)	2 (33.33)	1 (16.67)
	Tablets	49 (45.36)	29 (59.18)	20 (40.82)	0 (0.00)
	Other form	3 (2.78)	3 (100.0)	0 (0.00)	0 (0.00)
Price	<10	44 (40.74)	21 (47.73)	22 (50.00)	1 (2.27)
	10–20	23 (21.30)	14 (60.87)	9 (39.13)	0 (0.00)
	>20	41 (37.96)	24 (58.54)	16 (39.02)	1 (2.44)
Age group	Children	4 (3.70)	4 (100.00)	0 (0.00)	0 (0.00)
	Children and adults	20 (18.52)	6 (30.00)	13 (65.00)	1 (5.00)
	Adults	84 (77.78)	49 (58.33)	34 (40.48)	1 (1.19)
Country	Croatia	3 (2.78)	1 (33.33)	2 (66.67)	0 (0.00)
	Germany	6 (5.56)	4 (66.67)	2 (33.33)	0 (0.00)
	Japan	4 (3.70)	4 (100.00)	0 (0.00)	0 (0.00)
	Poland	81 (75.00)	42 (51.85)	38 (46.92)	1 (1.23)
	United States	8 (7.40)	3 (37.50)	4 (50.00)	1 (12.50)
	Other	6 (5.56)	5 (83.33)	1 (16.67)	0 (0.00)
	Several chemical forms	7 (6.48)	5 (71.43)	2 (28.57)	0 (0.00)
Chemical form	Calcium carbonate	72 (66.67)	41 (56.94)	29 (40.28)	2 (2.78)
	Calcium lactate	9 (8.33)	2 (22.22)	7 (77.78)	0 (0.00)
	Calcium salts of orthophosphoric acid	7 (6.48)	3 (42.86)	4 (57.14)	0 (0.00)
	Tricalcium phosphate	3 (2.78)	1 (33.33)	2 (66.67)	0 (0.00)
	Other forms	6 (5.56)	5 (83.33)	1 (16.67)	0 (0.00)
	No declaration	4 (3.70)	2 (50.00)	2 (50.00)	0 (0.00)
	Other	6 (5.56)	5 (83.33)	1 (16.67)	0 (0.00)
Declaration of the content	<100	16 (14.81)	11 (68.75)	5 (31.25)	0 (0.00)
	100–200	47 (43.52)	20 (42.55)	27 (57.45)	0 (0.00)
	>200	45 (41.66)	28 (62.22)	15 (33.33)	2 (4.45)
Type of food supplement	Single	13 (12.04)	5 (38.46)	8 (61.54)	0 (0.00)
	Complex	95 (87.96)	54 (56.84)	39 (41.05)	2 (2.11)

Another criterion for the division was the age of the patients for whom the FS were intended (Table 3). The smallest percentage of preparations on the market were supplements intended only for children; their Ca content was significantly lower compared to the Ca content in FS intended for both children and adults (89.6 vs. 259.8 mg/dose), as well as when compared to preparations which were dedicated only to adults (89.6 vs. 254.9 mg/dose).

The next division criterion was the country of origin of the producer producing a supplement (Table 4). The vast majority of supplements came from the domestic market ($n = 81$). The highest median Ca content was found in the preparations from Japan (596 mg/dose), but these values were not statistically significant.

Taking into account the chemical form, the highest percentage of FS contained Ca carbonate (72 out of 108 preparations). Supplements with this form had the highest median Ca content (335.4 mg/dose), but this difference was not statistically significant compared to other chemical forms (Table 5).

Supplements with a Ca content declared by the producers to be at the level of up to 100 mg/dose had a significantly lower median content compared to preparations with the content of 100 to 200 mg (95.3 vs. 208.2 mg/dose). The median Ca content for the preparations declared above 200 mg was 521.0 mg/dose (Table 6).

Preparations containing only one mineral component (Ca) were characterized by a higher median compared to multi-component preparations, but these differences were not statistically significant (Table 7). Taking the determined Ca content in individual FS and the number of servings recommended for consumption during the day, we calculated that patients can consume up to 130% less than the expected content (in this preparation, the declared value is 406 mg/dose, the marked value is 141 mg/dose, thus by consuming two doses a day, the patient consumes 530 mg less than they should) (Figure 1). Due to the very large dispersion and the need to improve the legibility of the figure, the preparation with the highest positive dispersion was symbolically marked as a 2000% dispersion but the actual dispersion was many times greater: the declared value is 0.12 mg/dose, while the marked value is 103 mg, which means that the patient takes as much as 85,653% more Ca than it appears from the declaration on the packaging.

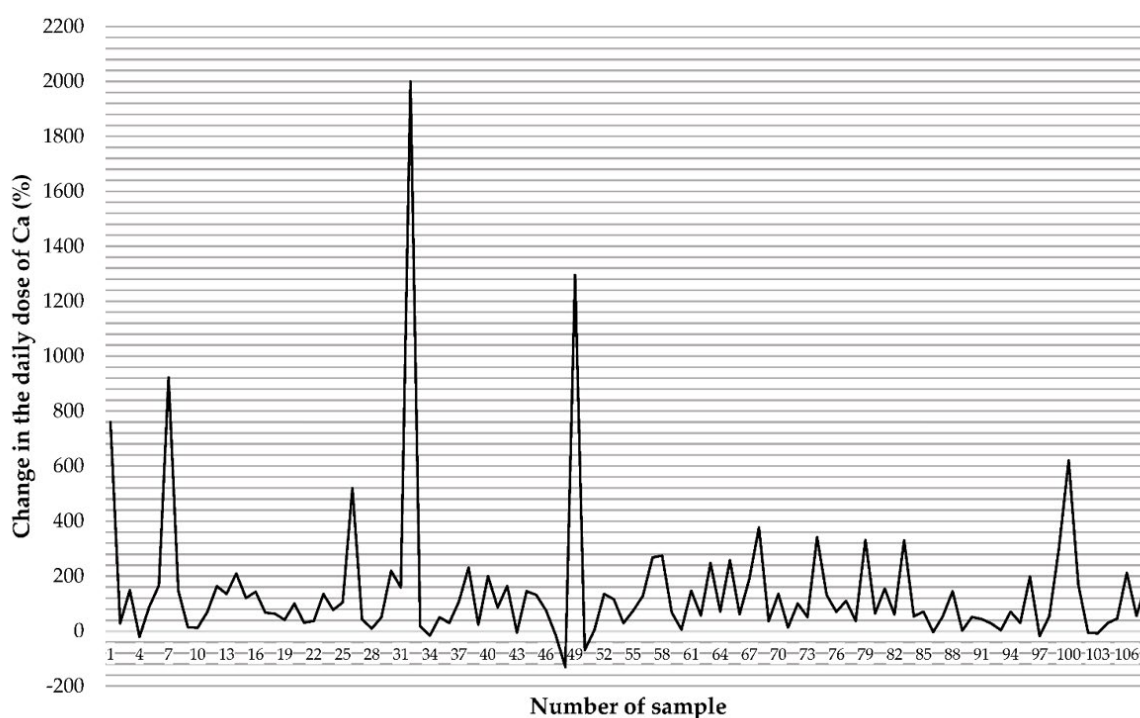


Figure 1. The disparity between the declared Ca content and that determined in food supplements based on the daily dose consumed.

The analysis of the Ca content in FS, in comparison to the applicable norms of the spread of the values of the results, showed that 1.9% of the samples had a too low Ca content in relation to the declaration, while in 54.6% of the supplements the content of the tested element was found to be too high.

We also assessed which subcategory of supplements was characterized by the highest percentage of samples with the standard (Table 8), and these were effervescent tablets (among all tested supplements, effervescent tablets within the normal range constituted 21.30% of the samples), with a price below PLN 10 (20.37%), for adults (31.48%), from Poland (35.18%), containing Ca carbonate (26.85%), with Ca content between 100 and 200 mg (25.00%), and complex preparations (36.11%).

3. Discussion

The research conducted as part of this project assessed the quality of 108 FS available for sale in Poland. These were preparations characterized by a different composition, price, pharmaceutical form or origin. The analysis of Ca content in one portion showed that most of the preparations contain more Ca than declared by the declarations placed by the producers on the packaging (54.6%). A small percentage of the samples was characterized by a Ca content lower than allowed by the standards (1.9%). We showed that 43.5% of the preparations were within the acceptable standards.

The problem of the difference between the value placed on the package and that determined by analytical methods in FS containing Ca has not been discussed in scientific publications so far; therefore, the following considerations are speculative.

A Ca content that is higher by several dozen or several hundred percent in one portion of the FS compared to the declared value, if used chronically, may lead to side effects. A retrospective study by Machado et al. in 2015 aimed at assessing the frequency of hypercalcemia associated with the use of supplementation with Ca supplements. Hypercalcemia was found in 429 patients over 18 years of age who were admitted to hospital between 2010 and 2013. The study excluded those patients whose hypercalcemia was caused by primary hyperparathyroidism, cancer, sarcoidosis or other diseases. Of 72 patients with hypercalcemia unrelated to PTH, 15 (20.8%) met the criteria for the diagnosis of Ca supplementation syndrome (renal failure, metabolic alkalosis, elevated serum bicarbonate and taking Ca/vitamin D supplements). Out of these 15 patients, 12 people reported complaints and symptoms, the cause of which was hypercalcemia (weakness, polyuria, abdominal pain, constipation, mental disorders and musculoskeletal discomfort). All patients received intravenous hydration, discontinued FS, and six patients received pharmacological treatment that lowered Ca levels in the form of calcitonin or zoledronic acid. Supplementation of Ca and vitamin D is considered beneficial for general health and prophylaxis, and also advised in the treatment of osteoporosis and vitamin D deficiency. However, it should be emphasized that the indiscriminate use of Ca and vitamin D supplements may have numerous health consequences [18]. The literature describes the case of a 36-year-old woman who developed severe hypercalcemia a few days after starting to breastfeed her second child. During and after pregnancy, the woman supplemented her high-calcium diet with moderate amounts of Ca carbonate to avoid an osteoporotic fracture that she had experienced while breastfeeding her first child. Metabolic changes occurring during lactation predispose women to hypercalcemia, therefore the recommended daily intake of Ca during breastfeeding should not be exceeded [19].

The highest Ca content determined in one portion of the FS is 2610 mg, while the maximum Ca content in the recommended daily portion in FS dedicated to adults is 1500 mg in accordance with the Resolution of the Team for Diet Supplements [14]. All other FS delivered less than 1500 mg per serving.

The European Food Safety Authority (EFSA) determined the population reference intake for Ca women and men aged 25 to 50 to be at the level of 950 mg [20]. Research published in 2017 indicated that the average Ca intake is 800–900 mg in Poland, 900–1000 mg in Croatia, and over 1000 mg in Germany [21], for example. Our research has shown

that the most popular pharmaceutical form of Ca present on the market are tablets. The average content in 1 serving (1 tablet) is 498.8 ± 407.0 mg. Assuming that Polish residents will use 1 tablet each, their Ca intake will increase to 1300–1400 mg, and in the case of German residents it will be at least 1500 (Figure 2). It should be emphasized that for some supplements it is recommended to take at least 2 tablets a day. A separate issue is Ca intake in children. For example, the EFSA recommendation for children aged 4–5 is 800 mg. The average content of children’s supplements was 105.5 ± 88.0 mg. In the case of this group, all preparations were characterized by a lower Ca content than the declared value.

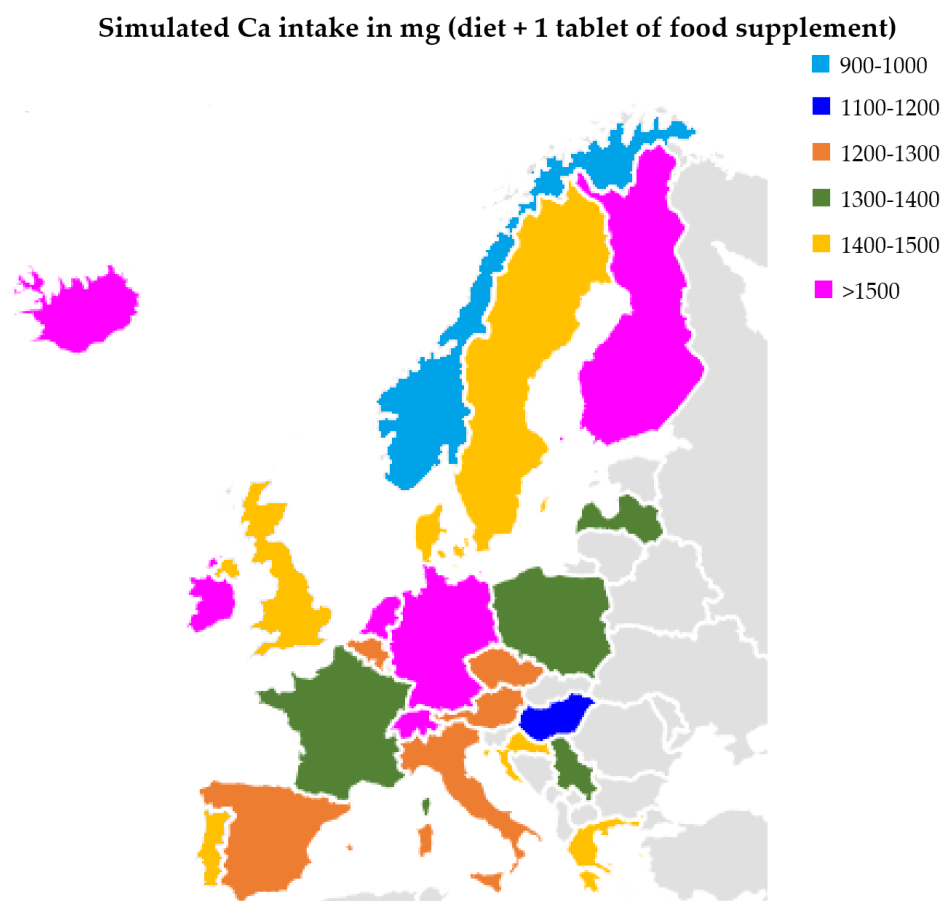


Figure 2. Calcium intake in selected European countries (with diet and 1 tablet of Ca).

Patients expect health benefits when buying FS and compliance of the declared value on the packaging with the actual content in the accepted FS. In the case of a deficiency diet supplemented with a preparation with a low content of Ca, nails may become brittle, the skin may become dry, and confusion can occur among the elderly. In turn, hypercalcemia can result in constipation, kidney stones and mood changes. Although the considerations regarding the Ca content in FS to predict health outcomes in the population are speculative, they constitute an important area of research.

The requirement for Ca varies depending on the age group. The highest amounts should be consumed during the period of rapid growth and puberty—the norm for girls and boys aged 10 to 18 at the estimated average requirement (EAR) level is 1100 mg/day—and in the elderly (1000 mg/day for people over 66 years of age). Studies have not found any additional benefits of consuming Ca in amounts greater than the recommended values [22]. Of the FS tested, 7.4% contained more than 1000 mg Ca/portion.

The average dietary intake of Ca in adults is at the level of 60% of the normal coverage [22]. It is related to the low absorption of Ca from diet (about 25%). Ca is best absorbed from milk and its products, while the lowest absorption of Ca is characteristic of plant

products, mainly due to the presence of oxalic acid and phytic acid. Additionally, the intake of this element from diet is hindered by the presence of insoluble fractions of dietary fiber, fat and too high phosphorus content [23]. If it is not possible to cover the demand for this element with the diet, it is recommended to use supplementation.

Ca deficiency leads to, among other things, an increased risk of osteoporosis. Other health consequences include increased excitability of the organism, tetany, neurological disorders and an increase in blood pressure [24]. Therefore, more and more people are supplementing Ca deficiencies with FS. However, it should be emphasized that, compared to drugs, FS are not subject to sufficient control. There are no legal regulations that require testing of the quantitative and qualitative composition of FS before being approved for sale. These preparations are subject to standard food control tests, but only after they have been released for sale and to a limited extent. This means that consumption of a food supplement, despite the declared content of minerals, does not ensure that the potential deficiencies in the diet will be supplemented [11]. Moreover, the tolerance limits for FS, taking into account the uncertainty in the measurement, are quite wide: for vitamins it is from -20% to $+50\%$, and for minerals from -20% to $+45\%$ [1].

The current norms regarding the recommended daily intake of Ca for the population of Poland over 19 years of age require the consumption of 1000 mg of this ingredient [22]. According to a study conducted by EFSA in 2015, the average Ca intake among people over 18 in nine European countries ranged from 690 mg to 1122 mg per day [25]. An American study covering the years 2009–2010 showed that the daily Ca intake among people aged 20–39 was 1210 mg for men and 947 mg for women [26]. In contrast, a study conducted in Poland in the years 2003–2005 on 1855 people aged from 20 to 34 showed that the average daily consumption of Ca in this group was 696 mg in men and 518 mg in women [27]. According to the results of a study from 2013–2014, which was also carried out in Poland on a group of people over 20 years of age, the average consumption of Ca for men was 586 mg/day and for women was 523 mg/day [28]. The above-mentioned examples show that the consumption of Ca in the Polish population is insufficient. In the case of insufficient amounts of this element in the diet, supplementation with appropriately high-quality preparations seems necessary.

Slightly different conclusions were formulated based on the assessment of Ca intake, carried out in a group of women ($n = 593$) aged 18–50 practicing sports at least twice a week. The standard for Ca was 800 mg. The median intake of Ca in the study group was 502 mg/day. As many as 92.0% of the respondents had a consumption of this ingredient below the norm at the EAR level. The authors estimated that 13.1% of women used Ca supplementation, but as many as 11.5% did not need an additional supply of this important mineral [29].

Over the years, many papers have been published, including systematic reviews and meta-analyses, evaluating the role of Ca for example in prevention of colorectal cancer [30], osteoporosis [31] and cardiometabolic disorders [32].

Currently, the expert consensus on Ca concerns, *inter alia*, reveals the following issues: supplementation with Ca and vitamin D results in a slight reduction in the risk of fractures, while supplementation with Ca alone is not effective. However, intervention at the level of whole populations has not been shown to be an effective strategy from a public health perspective. It is emphasized that side effects resulting from Ca supplementation may include the occurrence of gastrointestinal symptoms and kidney stones, while the issue of an increased risk of cardiovascular events requires further research. It should be added that supplementation with vitamin D alone is more effective in reducing the risk of falls. Contemporary recommendations indicate the need for calcium and vitamin D supplementation in patients at high risk of deficiency of these components and in patients treated for osteoporosis [33].

In the case of health consequences resulting from improper supplementation, pharmacokinetic factors should also be considered. Ca can be absorbed from the intestinal lumen into the blood by transcellular and paracellular routes. The transcellular pathway is an

active process, taking place, for example, in the duodenum and jejunum. The paracellular pathway is a passive process that occurs in the ileum and jejunum [34] among others.

Changes in the bioavailability of Ca during food storage were assessed, among others, in Ca compounds added to lemon juice. The highest bioavailability at the start of the study and after 6 months was found in calcium amino acid chelate ($45.09 \pm 0.59\%$ and $45.57 \pm 2.12\%$, respectively), Ca pidolate ($38.09 \pm 0.28\%$ and $34.38 \pm 1.13\%$, respectively), Ca lactate ($32.4 \pm 2.17\%$ and $32.68 \pm 1.27\%$, respectively) and Ca triphosphate ($31.21 \pm 4.43\%$ and $30.48 \pm 0.32\%$, respectively) [35].

A puzzling aspect is why manufacturers add higher Ca content to FS than declared. It seems necessary to introduce routine analysis of preparations, because currently this issue is not sufficiently controlled. As emphasized above, long-term supplementation with preparations with a higher content of Ca than the declared value can sometimes be harmful.

Among the limitations of this study, the following aspects could be mentioned: the quality assessment was carried out on the most popular preparations available for sale in pharmacies; therefore, the quality of preparations available, for example, in shops, herbal medicine shops, etc., may be different. In addition, the advantage of certain categories may also be indicated as a limitation, and not a homogeneous distribution, e.g., by far the largest percentage was for effervescent tablets and tablets, preparations typically aimed at adults or containing Ca carbonate in the composition. Further research should focus on the assessment of the bioavailability of the various chemical forms of Ca, and on the assessment of the concentration obtained after consumption of FS. Moreover, it seems necessary to evaluate the changes in Ca content during FS storage. In addition, due to the need to develop research on calcium absorption from FS, this discussion is speculative and indicates the direction of further analyses.

4. Materials and Methods

4.1. Materials

FS were selected on the basis of a review of the assortment available in stationary and online pharmacies, both in private pharmacies and chain pharmacies popular throughout the country. In total, 108 FS were included in the study, differing in pharmaceutical form, chemical form, producer's country of origin, price, composition, Ca content declared by the producer and the age group to which they are intended. Three sub-samples were taken from each supplement, from three different batches.

4.2. Sample Preparation

FS were homogenized in a vibrating crusher (Testchem, Radlin, Poland), and about 0.3 g, with an accuracy of 0.001 g, of the obtained powder was weighed into Teflon vessels. Then, 4 mL of spectrally pure concentrated (69%) nitric acid (Tracepur, Merck, Darmstadt, Germany) was added to the vessels. The microwave mineralization process was carried out in a closed system (Berghof, Speedwave, Eningen, Germany), and the analytical program is characterized in Table 9. The mineralizates were transferred to polypropylene vessels using deionized water, and the final weights were noted.

Table 9. Conditions for the mineralization of food supplements.

Steps	Temperature (°C)	Time (min)	Pressure (atm)	Microwave Power (%)
1	170	10	20	80
2	190	10	30	90
3	210	18	40	90
4	50	10	40	0

4.3. Determination of Ca Content

The content of Ca in FS was assessed by atomic absorption spectrometry (AAS) with Zeeman background correction at a wavelength of 422.7 nm using the Z-2000 instrument (Hitachi, Tokyo, Japan) with acetylene–air flame atomization. Lanthanum chloride (1% LaCl_3 , Sigma-Aldrich, Merck, Darmstadt, Germany) was applied as a masking reagent. On the basis of the calibration curve made from an absorbance–Ca concentration system, the concentration of Ca in the tested samples of FS was calculated.

In order to assess the accuracy of the method, a control was performed on the certified reference material Simulated Diet D (LIVSMEDELS VERKET, National Food Administration, Uppsala, Sweden), and six determinations of Ca were carried out. The range of certified values was 473–547 mg/kg, and all results were obtained within the acceptable range. The coefficient of variation (V) and the accuracy (% of error) was 0.96% and 0.22%, respectively.

4.4. Statistical Analysis and Interpretation of Results

The Statistica 13.3 software (Tibco, Palo-Alto, Santa Clara, CA, USA) was used to conduct statistical analyses. The results are presented in the tables as the mean with standard deviation ($\text{Av.} \pm \text{SD}$), minimum and maximum (Min-Max) values, the median (Med), the lower and upper quartile (Q1–Q3) and the interquartile range (IQR). The normality of the distribution of numerical data was evaluated using the Shapiro–Wilk, Kolmogorov–Smirnov and Lilliefors tests. Statistically significant differences between the groups were assessed using the Kruskal–Wallis ANOVA test and the Mann–Whitney U test, the level of significance was $p < 0.05$.

The obtained results regarding the Ca content in one dose of FS (e.g., in 1 tablet, capsule, etc.) were benchmarked to the recommendations of the European Commission from 2012 on establishing tolerance limits for minerals specified on the labels of FS. The Ca content of the preparation may be up to 20% lower and up to 45% higher than the declared value [16,17].

5. Conclusions

The conducted research has shown that more than half of Ca-containing food supplements are characterized by too high a content of this element; that is, they contain over 45% more than declared by the manufacturers. The universality of the use of supplementation among various age groups indicates that the quality of these preparations must be sufficiently high, and in accordance with the manufacturers' declarations.

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Publikacja H10



Mercury Content in Dietary Supplements From Poland Containing Ingredients of Plant Origin: A Safety Assessment

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Mercury (Hg) is a fairly common environmental pollutant. Chronic exposure to this element may cause, inter alia, kidney damage, and disturbances in the functioning of the nervous system. Literature data indicate that food, including dietary supplements (DS), may sometimes be contaminated with Hg. Therefore, the aim of the study was to assess Hg content in DS containing ingredients of plant origin. The study covered 200 DS available for sale in Poland. Hg content was determined by using the AAS method with the amalgamation technique using the AMA-254 analyzer. The highest average Hg content was found in preparations used as adjuncts for lowering glucose levels ($23.97 \pm 38.56 \mu\text{g/kg}$). The highest percentage of PTWI (1.143%) was found in DS aimed at improving vitality. Due to the fact that DS are commonly used, their quality should be constantly monitored.

Keywords: mercury, dietary supplements, herbs, food safety, PTWI

INTRODUCTION

Dietary supplements (DS) constitute a large group of food products. They are used by patients for prophylactic purposes, to support therapy or to supplement the diet with missing nutrients, and are sometimes treated as drugs (due to their similarity in terms of pharmaceutical form). It is also commonly believed that DS have a natural composition, are not harmful, have no side effects, and cannot be overdosed. Since they are sold for example in the form of tablets, capsules, syrups, and lozenges, they tend to be mistaken for medical products. As a result, they are often used by people with weakened immunity, diseases of various systems, or confirmed vitamin and mineral deficiencies. Data show that the popularity of DS and their presence in the market are steadily increasing (The Law on Food and Nutrition Safety, 2006; Main Sanitary Inspectorate, 2021).

Among DS available in Poland, the largest market share is held by preparations with magnesium (7.56%); immunostimulants (6.58%); probiotics (6.13%); supplements for strengthening bones, muscles, and joints (4.75%); vitamins and minerals for adults (4.65%); beauty supplements (4.40%); and food supplements with substances which improve vision (4.08%) (Dietary supplements, 2017).

The danger of using DS is related to the registration procedures, which are extremely straightforward. DS are not subjected to detailed quantitative or qualitative tests, confirming their high quality and safety. Unlike in the case of drugs, during the registration process, it is not necessary to prove that a supplement actually contains the ingredients that it claims to contain or

present the results of research on their safety (Regulation of the Minister of Health, 2011; Main Sanitary Inspectorate, 2021).

Mercury (Hg) is a major food contaminant. This toxic element can be released from primary natural sources (e.g., volcanic activity), primary anthropogenic sources (e.g., mining or natural gas extraction), or secondary anthropogenic sources (industrial processes). It is widely distributed in the environment; therefore, the general population is unable to avoid exposure to it (Vianna et al., 2019). Hg occurs mainly in three forms: elemental, inorganic (e.g., as mercury (I) chloride, mercury (II) chloride, or mercury (II) sulfide), and organic (methylmercury, dimethylmercury, ethylmercury, or phenylmercury) (EPoCitF, 2012). The abovementioned compounds are characterized by different bioavailability and toxic effects. The latter group includes methylmercury, which is the most common form of Hg in the food chain. For most people, the main source of exposure is diet, particularly one rich in fish and seafood (Communication from the Co, 2005; Richardson et al., 2011).

Chronic exposure to Hg can result in a number of health consequences, including disorders of the nervous system and kidneys (Johnson-Arbor et al., 2021; Novo et al., 2021). Metallic Hg enters the body *via* inhalation. Inorganic Hg compounds can mainly result in gastrointestinal disturbances and damage to the renal tubules, as well as the formation of free radicals that destroy DNA. Among the organic forms, methylmercury is most dangerous because of its high toxicity to humans. Approximately 95% of it is absorbed from food. Erythrocytes are the main accumulation site of methylmercury. Unfortunately, Hg can also penetrate the placenta and fetus and even cross the blood–brain barrier and the blood–cerebrospinal fluid barrier. Other health consequences include muscle weakness, peripheral vision disorders, problems with coordination of movements, and speech, hearing, and walking impairment (Bernhoft, 2012).

Contamination with this element has been highlighted in many reports (Kabata-Pendias, 2007; CD, 2008; Rice et al., 2014). However, it should be emphasized that there is low public awareness of the health consequences of excessive consumption of DS. Traditional herbal remedies from Asia (EPoCitF, 2012) may pose the greatest threat. It was shown that 17% of traditional herbal preparations exceeded the safety limits for Hg (Martena et al., 2010).

In 2008, the European Union set (Commission Regulation, 2008) the maximum permissible level of Hg in DS at 0.1 mg/kg. However, the European Commission Regulation (Commission Regulation, 2018) does not include data on Hg content in DS, which may indicate that this source presents a lower health risk than other food products listed in the regulation, for example, tree nuts (standard: 0.02 mg/kg), edible herbs and flowers (0.03 mg/kg), wild mushrooms (0.5 mg/kg), oilseeds (0.02 mg/kg), teas, coffee beans, or herbal infusions (0.02 mg/kg).

Our previous studies of 30 plant-based DS revealed contamination with Hg. The highest average content was found in DS supporting immunity (9.62–17.1 µg/kg) and those for the urinary system (9.98–21.2 µg/kg) (Socha et al., 2013). Alarming data were published in 2018. Hg content of 4212.04 µg/kg and 1806.12 µg/kg was detected in DS

containing the following ingredients of plant origin: bamboo shoots (85.72 mg/portion), horsetail (52.63 mg/portion), and algae *Chlorella pyrenoidosa* Chick (100% of algae, no specific data on the content) (Brodziak-Dopierała et al., 2018). The abovementioned data indicate that supplements containing herbs may still be a cause for concern and require strict control.

Therefore, the aim of our research was to evaluate the content of Hg in DS containing ingredients of plant origin. In addition, exposure indicators related to the regular use of Hg-contaminated DS were assessed, which made it possible to perform such a comprehensive assessment of exposure to the abovementioned element.

MATERIALS AND METHODS

Materials

In this research, 200 DS were included. All the analyzed products were available for sale in Poland. The study samples consisted of DS for the following: acne therapy support ($n = 6$), cholesterol control ($n = 7$), detoxification ($n = 5$), digestive tract support ($n = 21$), glucose level control ($n = 5$), for hair, skin and nails, called nutricosmetics ($n = 17$), immunity ($n = 18$), memory ($n = 9$), the nervous system ($n = 4$), sore throat ($n = 11$), the urinary tract ($n = 10$), for veins ($n = 6$), for vision and eye health ($n = 5$), vitality ($n = 17$), and supplements containing vitamins and minerals ($n = 23$) for weight loss ($n = 25$) and others ($n = 11$). All the studied DS contained ingredients of plant origin.

The DS were purchased in stationery and online drugstores belonging to nationwide pharmacy chains.

Methods

Preparation of DS for Analysis

Solid DS were homogenized in a vibrating mill (Testchem, Radlin, Poland), while liquid ones were mixed using a Vortex Mixer Benchmixer (Benchmark, Sayreville, NY, United States of America). The weighed samples (0.02 g or 50 µL, with an accuracy of 1 mg) were placed in a cuvette, and Hg content was determined.

Determination of Hg Content

The content of Hg was measured using atomic absorption spectrometry (AAS), using an Advanced Mercury Analyzer (AMA)-254 (Leco Corp. Altec Ltd. Prague, Czech Republic), according to the methodology described previously (Bielecka et al., 2020). This method facilitates the separation of Hg from its compounds, both inorganic and organic, and transforming it into an atomic form.

The process of determining the content of Hg consisted of 3 steps. The first step was to dry the sample and then burn it in an oxygen stream; medicinal oxygen was used as the carrier gas. The second step was to pass the released Hg vapor through the catalytic column; the vapors were captured by the amalgamator. The third step was to release Hg from the amalgamator and measure its content using the AAS method at a wavelength of 254 nm. The method's limit of quantification was 0.003 ng Hg/g sample.

Quality Control of the Method

Quality control of the method was performed using certified reference material – *Mixed Polish Herbs* (INCT-MPH-2), obtained from the Institute of Nuclear Chemistry and Technology (Warsaw, Poland). The particular analytical steps were analogous to the procedure for determining Hg content in the samples. The recovery rate was 102%, and the precision rate was 2.1%.

Comparison to the Norm

The obtained results were compared to the applicable Commission Regulation (Commission Regulation, 2008), establishing the maximum levels of certain contaminants in foodstuffs, according to which the maximum level of Hg in DS is 0.1 mg/kg.

Assessment of Consumption Safety

The risk of the health consequences related to the consumption of Hg in DS was estimated by calculating selected exposure indicators, such as the estimated daily intake (EDI), the estimated weekly intake (EWI), the percentage of provisional tolerable weekly intake (% PTWI), and Hg consumption during 1 month and 1 year. The EDI [$\mu\text{g}/\text{day}$] was calculated using the following formula:

$$EDI = C \times \text{Cons},$$

where C [$\mu\text{g}/\text{kg}$] is the concentration of Hg in the sample and Cons [kg] is the daily portion of the studied supplement, considering the weight of the portion and maximum daily dosage. The EWI [$\mu\text{g}/\text{week}$] was estimated by multiplying the EDI value by seven (which corresponds to 1 week). To determine the % PTWI [$\mu\text{g}/\text{kg}/\text{week}$], the following equation was used:

$$\%PTWI = \left[\left(\frac{EWI}{BW} \right) / 4 \right] * 100,$$

where BW is the average body weight of an adult in Poland (the weight of 70 kg was assumed). The obtained results were compared to the norm established by the European Food Safety Authority at 4 $\mu\text{g}/\text{BW}/\text{week}$ (EPoCitF, 2012).

Moreover, the THQ index (target hazard quotient) was calculated for selected DS using the following formula:

$$THQ = \frac{Fr \times D \times \text{Cons} \times C}{RfD \times BW \times T} \times 10^{-3},$$

where Fr is the frequency of exposure [365 days/year], D is the time of exposure [70 years], Cons is the average DS consumption per day [g], C is the concentration of Hg in the DS [mg/kg], RfD is the oral reference dose [0.3 $\mu\text{g}/\text{kg}$ body weight/day], BW is the body weight [kg], and T is the time of exposure [365 days/year \times 70 years].

The interpretation of the THQ is as follows: if the THQ value is above 1, it may indicate a potential risk associated with the consumption of the heavy metal in question with the DS. On the other hand, when the value is below 1, it indicates a low non-carcinogenic risk.

The supplementation materials provide Hg content per portion (i.e., one capsule, one tablet, etc.), daily consumption (content in one portion multiplied by the number of portions recommended for consumption by the manufacturer), and weekly consumption – calculated analogously by multiplying the daily consumption for 7, monthly – for 30, and yearly – for 365.

Statistical Analysis of the Results

Data were analyzed using Statistica 13.3 (TIBCO Software Inc. Palo Alto, CA, United States). In order to assess the consistency of the data distribution with normal distribution, the Shapiro–Wilk, Kolmogorov–Smirnov, and Lilliefors tests were used. Due to the lack of normality in the data distribution, the Kruskal–Wallis analysis of variance (ANOVA) was performed to compare the content of Hg in individual categories and between pharmaceutical forms. The table lists the mean (X), standard deviation (SD), minimum (Min), and maximum (Max) levels to compare the results with the literature data, the median (Me), lower (Q1), and upper (Q3) quartile levels due to lack of normality of the data distribution. The level of statistical significance was set at $p < 0.05$.

RESULTS

Taking into account all studied DS ($n = 200$), the mean Hg content was $3.37 \pm 7.65 \mu\text{g}/\text{kg}$ and the median content was $1.69 \mu\text{g}/\text{kg}$, while the range of quartiles ranged from 1.10 to $2.86 \mu\text{g}/\text{kg}$.

Table 1 shows the content of Hg in the tested DS, with division into categories. Detailed data on Hg content in individual DS are included in the Supplementary Materials section: **Supplementary Tables S1–S17**.

In this study, the highest median Hg content ($5.94 \mu\text{g}/\text{kg}$) was detected in the group of supplements responsible for controlling glucose levels, while the lowest was in the detoxifying supplements ($0.64 \mu\text{g}/\text{kg}$). The average concentrations of Hg ranged from $0.23 \mu\text{g}/\text{kg}$ in a supplement for the digestive tract to $91.40 \mu\text{g}/\text{kg}$ in a product designed to control blood glucose levels.

Among the supplements supporting acne treatment, the highest content of Hg ($6.01 \mu\text{g}/\text{kg}$) was found in a supplement containing the extract of *Viola tricolor* L. (wild pansy) (100 mg).

In the case of DS used to help in reducing cholesterol levels, the highest content of Hg ($2.16 \mu\text{g}/\text{kg}$) was found in a supplement containing red yeast rice extract with monacolin K (250 mg) and phytosterols (47.5 mg).

Considering the subgroup comprising detoxifying supplements ($n = 5$), the maximum Hg concentration ($3.52 \mu\text{g}/\text{kg}$) was detected in products containing silver birch extract (600 mg), common dandelion extract (350 mg), *Orthosiphon spicatus* (Thunb.) Backer, Bakh. f. Steenis not Benth. extract (350 mg), ginseng root extract (300 mg), white nettle extract (300 mg), broadleaf plantain extract (250 mg), *Pilosella officinarum* Vaill. extract (250 mg), maypop extract (200 mg), fennel extract (175 mg), olive extract (175 mg), green tea extract (100 mg), and lemon extract (100 mg).

TABLE 1 | Hg content in DS and health risks of their use.

Category of the supplements	n	Content of Hg [μg/kg]		Indicators		Intake of Hg [μg] min-max		PTWI min-max [%]
		X ± SD min-max	Me Q ₁ -Q ₃	EDI [μg]	EWI [μg]	Monthly	Annual	
Acne	6	2.24 ± 1.95 0.71–6.01	1.83 1.06–2.17	0.001–0.003	0.004–0.021	0.015–0.088	0.186–1.069	0.001–0.007
Cholesterol control	7	1.29 ± 0.56 0.42–2.16	1.32 1.00–1.55	0.001–0.002	0.004–0.012	0.016–0.051	0.192–0.616	0.001–0.004
Detox	5	1.20 ± 1.30 0.49–3.52	0.64 0.65–0.68	0.001–0.017	0.004–0.117	0.017–0.502	0.206–6.109	0.001–0.042
Digestive tract	21	1.91 ± 1.66 0.23–7.10	1.42 0.91–2.14	0.000–0.014	0.001–0.097	0.002–0.418	0.029–5.082	<0.001–0.035
Glucose level	5	23.97 ± 38.56 0.80–91.40	5.94 0.99–20.72	0.001–0.119	0.004–0.830	0.018–3.558	0.223–43.283	0.002–0.296
Nutricosmetics	17	4.13 ± 5.32 0.56–22.00	2.48 1.20–3.72	0.000–0.068	0.003–0.474	0.013–2.030	0.162–24.693	0.001–0.169
Immunity	18	2.62 ± 2.73 0.34–11.88	1.90 0.90–2.71	0.001–0.047	0.005–0.327	0.022–1.399	0.263–17.025	0.002–0.117
Memory	9	1.45 ± 0.98 0.66–3.79	1.46 0.92–2.69	0.000–0.006	0.003–0.041	0.012–0.177	0.147–2.159	0.001–0.015
Nervous system	4	2.41 ± 1.64 0.80–4.64	2.09 1.49–3.01	0.002–0.009	0.011–0.065	0.046–0.277	0.564–3.371	0.004–0.023
Throat	11	1.54 ± 0.87 0.27–3.20	1.50 1.12–1.99	0.003–0.050	0.018–0.352	0.078–1.510	0.948–18.371	0.006–0.126
Urinary tract	10	4.49 ± 4.90 0.67–13.84	1.99 1.37–7.84	0.001–0.012	0.005–0.082	0.019–0.350	0.236–4.264	0.002–0.029
Veins	6	4.50 ± 2.49 1.27–8.57	3.76 3.66–5.44	0.001–0.011	0.005–0.076	0.021–0.324	0.252–3.947	0.002–0.027
Vision	5	6.06 ± 8.91 1.27–21.81	1.39 1.38–4.46	0.001–0.018	0.004–0.126	0.016–0.538	0.196–6.544	0.001–0.045
Vitality	17	2.06 ± 1.38 0.46–5.32	1.81 1.11–2.65	0.000–0.457	0.003–3.201	0.014–13.720	0.172–166.932	0.001–1.143
Vitamins and minerals	23	4.21 ± 8.61 0.57–42.96	1.86 1.18–3.35	0.001–0.036	0.006–0.252	0.026–1.079	0.312–13.124	0.002–0.090
Weight loss	25	3.08 ± 3.62 0.52–17.26	1.78 1.21–2.88	0.001–0.026	0.004–0.184	0.018–0.790	0.215–9.617	0.001–0.066
Other	11	1.56 ± 0.69 0.54–2.47	1.36 1.09–2.29	0.000–0.023	0.001–0.163	0.006–0.699	0.070–8.509	<0.001–0.058

X-average, SD-standard deviation, Me-median, Q₁-quartile 1, Q₃-quartile 3, EDI-estimated daily intake, EWI-estimated weekly intake, PTWI-provisional tolerable weekly intake.

Among the DS recommended to improve digestive tract function ($n = 21$), the greatest amount of Hg (7.10 μg/kg) was detected in a sample containing mint leaves.

The next studied subgroup included supplements for glucose level control ($n = 5$). In this subgroup, one sample with the highest Hg concentration, among whose ingredients were extracts of *Gymnema sylvestre* R. Br. and *Trigonella foenum graceum* L. (fenugreek), contained the highest level of detected Hg, which was nearly the maximum permissible amount of Hg (91.40 μg/kg).

In the subgroup of supplements with bioactive substances, which could play a role in improving the condition of hair, skin, and nails ($n = 17$), the highest level of Hg (22.00 μg/kg) was observed in a sample containing *Chlorella pyrenoidosa* Chick (200 mg).

Analyses of Hg content in the subgroup of supplements recommended to strengthen the immune system ($n = 18$) revealed the highest amount of the element (11.88 μg/kg) in a sample containing the extract of acerola.

Among the DS recommended for memory support ($n = 9$), the highest level of Hg (3.79 μg/kg) was recorded in supplements with extracts of *Panax ginseng* C.A. Meyer (71.43 mg), *Ilex paraguariensis* A. St.-Hill (150 mg), and *Bacopa monnieri* (L.) Wettst (36 mg).

In the case of supplements supporting the functioning of the nervous system ($n = 4$), the greatest Hg level (4.64 μg/kg) was found in a sample containing lemon balm leaf.

In our study, the highest Hg (3.20 μg/kg) concentration in the analyzed DS for patients with throat symptoms ($n = 11$) was observed in products containing extracts of *Salvia officinalis* L. (11.25 mg), *Althaea officinalis* L. (11.25 mg), *Tilia cordata* Mill (10 mg), *Matricaria recutita* L. (8 mg), propolis (5.25 mg), *Sambucus nigra* L. (3.75 mg), and *Thymus vulgaris* L. (6.25 mg).

Ten of the tested products were dedicated to supporting the urinary tract. In this subgroup, the highest content of Hg (13.84 μg/kg) was detected in DS based on cranberry fruit extract (360 mg).

The maximum Hg content in the next studied group, that is, supplements to improve the condition of veins ($n = 6$) was 8.57 μg/kg. These DS were based on *Vitis vinifera* L. leaf extract (84 mg), and grape seed extract (79 mg).

In the subgroup of supplements claiming to support vision ($n = 5$), the highest level of Hg (21.81 μg/kg) was found in supplements containing bilberry fruit extract (290 mg) and Aztec marigold flower extract (15 mg).

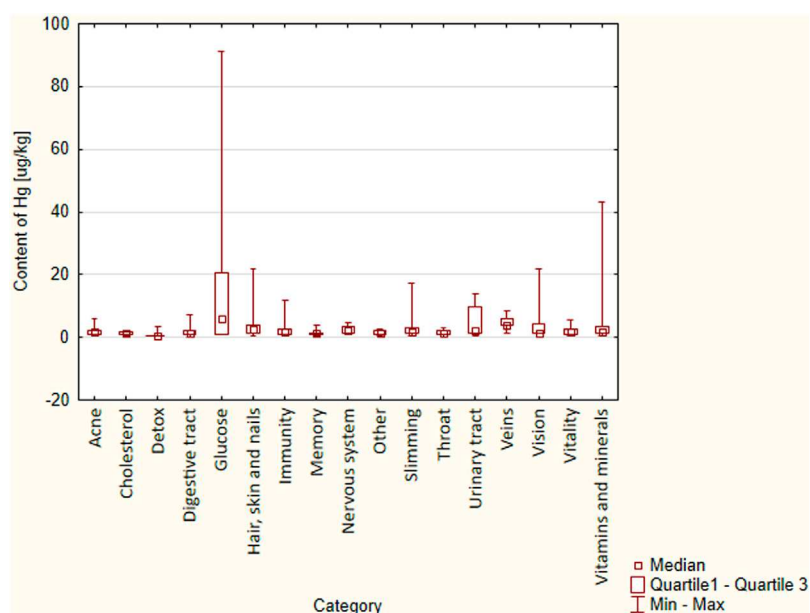


FIGURE 1 | Differences in Hg content between the category of DS ($p = 0.068$, results were not statistically significant).

Among the DS recommended to improve vitality ($n = 17$), the highest content of the tested toxic element ($5.32 \mu\text{g/kg}$) was found in a sample based on *Ginseng* extract C.A. Meyer (50 mg) and ginkgo extract (40 mg).

In the next studied subgroup—DS containing vitamins and minerals—the highest concentration of Hg ($42.96 \mu\text{g/kg}$) was detected in a supplement containing extract of *Withania somnifera* (L.) Dunal (80 mg).

The largest surveyed group ($n = 25$) included products designed to promote weight loss. The highest concentration of Hg ($17.26 \mu\text{g/kg}$) was observed in one sample containing the following substances: extract of *Camellia sinensis* (L.) Kuntze (105 mg), extract of *Zingiber officinale* Rosc (100 mg), extract of cayenne pepper (70 mg), extract of green coffee (5 mg), extract of *Cinnamomum* Scheffer (5 mg), extract of *Paullinia cupana* Kunth (6.6 mg), extract of *Citrus sinensis* (L.) Osbeck, extract of *Citrus grandis* Osbeck, and extract of *Citrus aurantium vel. dulcis* L. (10 mg).

In our research, 11 products were not classified into any of the studied subgroups. The highest Hg content ($2.47 \mu\text{g/kg}$) was found in a product containing extracts of *Melissa officinalis* L. leaves (300 mg), *Humulus lupulus* L. (100 mg), and *Rhodiola rosea* L. (100 mg).

The study showed that the examined categories of DS did not differ significantly in terms of Hg content ($p = 0.068$) (**Figure 1**).

Moreover, it was assessed that the pharmaceutical form of the DS affected the content of the tested element— $p = 0.045$ (**Figure 2**). The preparations available in the form of sachets for infusion were characterized by the highest median: $2.66 \mu\text{g/kg}$ (Q1-Q3: $1.36\text{--}5.49 \mu\text{g/kg}$).

Our analyses showed that the content of Hg in all tested DS was below 0.1 mg/kg . In the case of one DS, the content of this element was above 0.09 mg/kg , which is a value close to the maximum allowable concentration.

The calculated percentage of the PTWI for Hg due to intake of studied DS is included in **Table 1**. The lowest percentage of the PTWI was lower than 0.001% , while the highest percentage of the PTWI was calculated for one DS designed to boost vitality (1.143%) (Table S14). Generally, the values of this indicator in the vast majority of samples were lower than 1% , ranging from 0.001 to 0.030% .

In the case of the DS with the highest content per sample (category: glucose, $91.40 \mu\text{g/kg}$) and the one with which the most Hg would be consumed during a single day (category: vitality, $22.00 \mu\text{g/kg}$, it is recommended to consume 15 servings per day), the THQ index was calculated as follows: $4.10\text{E-}03$ and $3.22\text{E-}03$, respectively.

DISCUSSION

DS are used by consumers from different age groups. Due to their similarity to medicinal products, they are applied in the treatment of various diseases. For the abovementioned reason, they should be of high quality. Registration procedures and national regulations do not require qualitative research; therefore, this kind of research is of interest to various authors.

Our research has shown that the highest average Hg content was found in DS used for lowering glucose levels ($23.97 \pm 38.56 \mu\text{g/kg}$). The supplement with the highest concentration of the element ($91.40 \mu\text{g/kg}$) contained two ingredients of plant origin, namely, extract of *Gymnema sylvestre* R. Br (185 mg) and extract of *Trigonella foenum graecum* L./fenugreek (90 mg). Moreover, they contain chromium and lipoic acid as well.

It can be assumed that high concentration of Hg in *Gymnema sylvestre* R. Br. may stem from the fact that the plant is mainly grown in Asia. China is a country where Hg is the most prevalent toxic element (Huang et al., 2022); hence, plants are likely to absorb it.

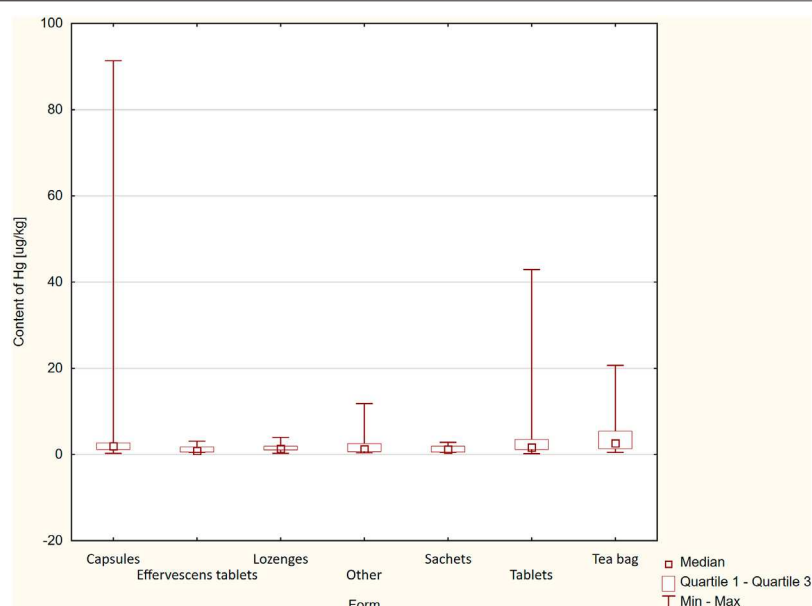


FIGURE 2 | Differences in Hg content between forms of DS ($p = 0.045$).

To our knowledge, the research conducted in this project involved more DS than in most previous publications to better assess consumer exposure. Research on Hg content in 24 DS containing ingredients of plant origin was carried out by Brodziak-Dopierała et al. (Brodziak-Dopierała et al., 2018). The authors measured the content of Hg using the same method that we did and found that the studied supplements contained 0.02 to 4,293.07 µg/kg of Hg. The average concentration was almost 58 times higher than that shown in our study (193.77 vs 3.37 µg/kg). The second highest result (1806.12 µg/kg) involved a DS containing *Chlorella pyrenoidosa* Chick algae. In our study, a *Chlorella*-based product with the recommended dose of as many as 15 tablets/day also proved to have one of the highest amounts of Hg (22.001 µg/kg). The result obtained by us, however, was as much as 82 times lower than the highest one in the abovementioned studies. Moreover, the authors showed that the preparations in tablets were characterized by a significantly higher mean Hg content compared to capsules (274.80 ± 917.64 µg/kg vs 5.95 ± 7.30 µg/kg). Our research looked at more pharmaceutical forms and revealed that the infusion bags had the highest median Hg content.

Chlorella has good Hg absorption properties. It prevents the reabsorption of Hg from the gastrointestinal tract; therefore, it can be used as an effective absorbent to remove Hg from the body (Yadav et al., 2020). In a study conducted by Caldas et al., Hg was detected in all samples, while none of the samples exceeded the acceptable limit (from <0.01 to 0.09 µg/g) (Caldas and Machado, 2004). On the other hand, in a study analyzing the Polish supplement market, a preparation based on *Chlorella* had one of the highest concentrations of Hg: 1810 µg/kg, which exceeded the acceptable standard (100 µg/kg) (Brodziak-Dopierała et al., 2018).

Other data, including Hg content in 24 DS, come from Mexico. The content of the discussed element ranged below 240–850 µg/kg. According to the authors, Hg at the detection

limit level was present in only 5 DS (about 21% of samples). The highest content (850 µg/kg) was found in a DS containing Guaco stem (*Mikania guaco* Bonpl), red vine leaves (*Vitis vinifera* L.), horse plant (*Equisetum arvense* L.), and gorongoro bark (García-Rico et al., 2007). Hg content in DS can be explained by the presence of this ingredient in the form of cinnabar (HgS), especially in Chinese preparations, including those used for the treatment of throat diseases (Bin et al., 2001; Wu et al., 2002).

Studies assessing the quality of 49 pharmaceutical products from Korea containing raw materials of plant origin showed that the content of total Hg in these preparations was high. For example, the highest amount was found in a preparation containing royal jelly—as much as 159.89 µg/kg—which is about 1.76 times higher than the highest result obtained by us. The mean methylmercury content of the herbal preparations in this study was 31.18 µg/kg, while the preparations containing *Spirulina* had 0.62 µg/kg of Hg (Lee and Lee, 2013).

Another study of DS from Poland assessed the quality of 33 products containing macro and microelements ($n = 7$), vitamins ($n = 5$), and nutricosmetics ($n = 6$) and classified as “other” ($n = 15$). The average content of Hg was 5.5 µg/kg, with the highest in a preparation containing vitamin C and rutin (16.7 µg/kg) (Kowalski and Frankowski, 2015).

Another study of DS from Poland involved a quality assessment of 41 DS containing terrestrial plants and microalgae. The authors showed that 29.3% of the investigated DS were contaminated with Hg. The average content of Hg in the products containing ingredients of plant origin was 5 ± 8 µg/kg, while in those based on microalgae — 3 ± 6 µg/kg. The highest concentration (28 µg/kg) was found in tablets which contained *Rehmannia glutinosa* (Gaertn.) Steud. radix and Wolfiporia (Ćwiela-Drabek et al., 2020).

The prevalence of Hg contamination in Ayurvedic herbal DS was demonstrated by Mikulski et al. (Mikulski et al., 2017). The

presence of Hg was detected in as many as 38% of the tested preparations. It is very disturbing that the content of the toxic element ranged from 800 to 279,000 µg/kg. Brihat Vatchintamani Ras (139,500 µg/0.5 g pill) was characterized by the highest content of Hg per one pill.

In contrast, studies of 10 DS from Turkey, carried out using the ICP-OES method, showed no Hg in the tested preparations (Canbay and Doğançtürk, 2017).

The presence of Hg in DS containing ingredients of plant origin can be explained by the fact that plants are one of the best agents for removing Hg²⁺ + impurities from soil. Bioabsorption is based on mechanisms such as chelation, ion exchange, and species of the structural polysaccharide cell wall network absorption by physical forces and ion entrapment in inter- and intra-fibrillar capillaries. For example, Hg is selectively accumulated by *Carica papaya* L. wood or *Ricinus communis* L. (Basha et al., 2009; Kumar et al., 2017). It should be emphasized that the absorption of Hg may be toxic not only to humans but also to plants themselves.

DS can be one of the sources of exposure to Hg. Other sources of exposure to organic forms of Hg include fossil fuel emissions, medical waste incineration, dental amalgams, and various other products, including skin creams, bactericidal soaps, teething powders, painkillers, thermometers, blood pressure gauges, barometers, bulbs, and batteries. Other sources of organic Hg include phenylmercury and ethylmercury compounds, which used to be components of latex paints before the 1990s, and thimerosal, which was used as a preservative in vaccines (Rice et al., 2014).

Chronic exposure to Hg may result in, inter alia, disruption of the endocrine system. Hg is mainly stored in the thyroid and pituitary gland. Previous research has shown that the concentration of the element in these organs ranged from 6.3 to 77 ng/g, while in another study, it amounted to 28 ng/g. These levels exert neurotoxic and cytotoxic effects (Rice et al., 2014). Exposure to Hg can cause changes in the nervous system, which is associated with a toxic increase in reactive oxygen species (Fernandes Azevedo et al., 2017). However, the THQ index calculated by us does not indicate an increased non-carcinogenic risk resulting from consuming the DS under investigation.

In another study, the DS which had the highest % PTWI (3.91%) contained two ingredients of plant origin: extract of millet (50 mg) and extract of wheat germ (50 mg) (Brodziak-Dopierała et al., 2018). The highest % PTWI calculated in our study amounted to 1.143% and was detected in a preparation containing *Guarana* Kunth seed extract.

Hg, along with several other elements (Cd and Pb), has been recognized as an impurity arising from the food chain (Tchounwou et al., 2012), which is also confirmed by our study. Among the many factors affecting the concentration of Hg in food are natural factors, including not only growing conditions (type of water and soil) and cultivation practices but also meteorological conditions (i.e. geological areas for Hg-rich formations and atmospheric deposition rate). The fact that supplement ingredients are often plant-based may account for Hg contamination. Plants quite easily absorb heavy metals from soil and water, and these can remain in the final product, even after processing. It should also be emphasized that the

location of the source of raw materials for the production of supplements is of considerable importance and may directly affect the content of Hg in the final product (Bandara et al., 2020).

Summing up, although the content of Hg in the studied DS was lower than that reported in most of the literature and did not exceed the permissible maximum content prescribed by law, it should be emphasized that since Hg is a toxic element, any amount of it may be harmful to health. During the production of DS, strict procedures for obtaining raw materials from crops controlled in terms of environmental pollution, including soil, as well as procedures for cleaning plant materials and eliminating the risk of contamination of the final product at all stages should be implemented.

CONCLUSION

DS containing ingredients of plant origin are mostly safe in terms of Hg content, but it should be stressed that Hg is a highly toxic element, and its long-term use may pose a health hazard. This is especially dangerous in the case of chronically ill people who use several DS at the same time. Consumers and pharmacists should pay attention to the origin of DS and the recommended number of tablets taken during the day, as in some cases, higher doses can lead to increased exposure to Hg. In addition, there is a recognized need for DS to be tested for quality and safety before being placed on the market.

DATA AVAILABILITY STATEMENT

The results of the research carried out may be available from the authors. Requests to access the datasets should be directed to anna.puscion-jakubik@umb.edu.pl.

AUTHOR CONTRIBUTIONS

Conceptualization, KS, RM-Ż, and AP-J; methodology, AP-J and KS; software, AP-J, AM, DA, MI, MG, and JB; formal analysis, AP-J, KS, and RM-Ż; data curation, DA, MI, AM, MG, JB, and AP-J; writing—original draft preparation, AP-J, MG, and JB; writing—review and editing, KS and RM-Ż; visualization, AP-J; and supervision, KS and RM-Ż.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fphar.2021.738549/full#supplementary-material>

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Oświadczenia współautorów publikacji

H1

Białystok, 19.12.2022 r.

Dr hab. Katarzyna Socha
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OŚWIADCZENIE WSPÓŁAUTORA PUBLIKACJI

Oświadczam, że w publikacji:

Puścion-Jakubik A., Socha K., Borawska M.H. *Comparative study of labelled bee honey from Poland and the result of the melissopalynological analysis*. Journal of Apicultural Research 2020, 59(5), 928-938

mój udział polegał na recenzji finalnej wersji manuskryptu i sprawowaniu opieki merytorycznej.

Wyrażam zgodę na włączenie w/w publikacji do postępowania habilitacyjnego dr Anny Puścion-Jakubik.

KIEROWNIK
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Białystok, 19.12.2022 r.

Prof. dr hab. Maria H. Borawska
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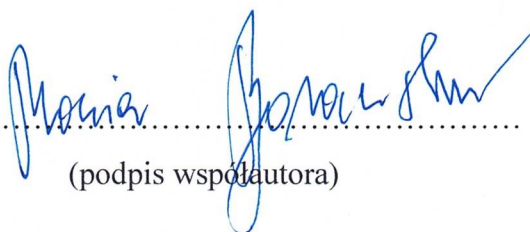
OŚWIADCZENIE WSPÓŁAUTORA PUBLIKACJI

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Puścion-Jakubik A., Socha K., Borawska M.H. *Comparative study of labelled bee honey from Poland and the result of the melissopalynological analysis*. Journal of Apicultural Research 2020, 59(5), 928-938

mój udział polegał na współtworzeniu koncepcji publikacji i recenzji finalnej wersji manuskryptu.

Wyrażam zgodę na włączenie w/w publikacji do postępowania habilitacyjnego dr Anny Puścion-Jakubik.



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Oświadczenia współautorów publikacji

H2

Białystok, 19.12.2022 r.

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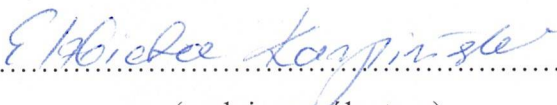
OŚWIADCZENIE WSPÓŁAUTORA PUBLIKACJI

Oświadczam, że w publikacji:

Puścion-Jakubik A., Karpińska E., Moskwa J., Socha K. *Content of phenolic acids as a marker of Polish honey varieties and relationship with selected honey-quality-influencing variables*. Antioxidants, 2022, 11(7), ID 1312

mój udział polegał na współtworzeniu koncepcji publikacji, wykonaniu oznaczenia chromatograficznego, w tym walidacji, obsłudze specjalistycznego oprogramowania, analizie uzyskanych danych i pisaniu części manuskryptu.

Wyrażam zgodę na włączenie w/w publikacji do postępowania habilitacyjnego dr Anny Puścion-Jakubik.


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Białystok, 19.12.2022 r.

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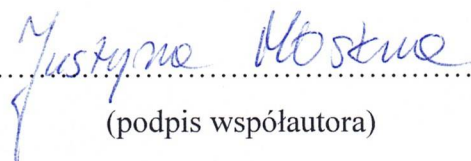
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mój udział polegał na pomocy w opracowaniu metodyki i analizie uzyskanych danych.

Wyrażam zgodę na włączenie w/w publikacji do postępowania habilitacyjnego dr Anny Puścion-Jakubik.

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Białystok, 19.12.2022 r.

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mój udział polegał na analizie uzyskanych danych, walidacji, analizie formalnej, recenzji finalnej wersji manuskryptu i sprawowaniu opieki merytorycznej.

Wyrażam zgodę na włączenie w/w publikacji do postępowania habilitacyjnego dr Anny Puścion-Jakubik.

KIEROWNIK
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Oświadczenia współautorów publikacji

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Białystok, 19.12.2022 r.

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OŚWIADCZENIE WSPÓŁAUTORA PUBLIKACJI

Oświadczam, że w publikacji:

Puścion-Jakubik A., Bielecka J., Grabia M., Markiewicz-Żukowska R., Soroczyńska J., Teper D., Socha K. *Comparative analysis of antioxidant properties of honey from Poland, Italy and Spain based on the declarations of producers and their results of melissopalinalological analysis*. Nutrients, 2022, 14(13), ID 2694

mój udział polegał na współprowadzeniu badań (wykonaniu oznaczenia całkowitej zawartości związków fenolowych), walidacji i analizie uzyskanych danych.

Wyrażam zgodę na włączenie w/w publikacji do postępowania habilitacyjnego dr Anny Puścion-Jakubik.

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OŚWIADCZENIE WSPÓŁAUTORA PUBLIKACJI

Oświadczam, że w publikacji:

Puścion-Jakubik A., Bielecka J., Grabia M., Markiewicz-Żukowska R., Soroczyńska J., Teper D., Socha K. *Comparative analysis of antioxidant properties of honey from Poland, Italy and Spain based on the declarations of producers and their results of melissopalinalological analysis*. Nutrients, 2022, 14(13), ID 2694

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Wyrażam zgodę na włączenie w/w publikacji do postępowania habilitacyjnego dr Anny Puścion-Jakubik.



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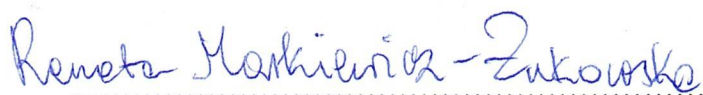
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mój udział polegał na pomocy w opracowaniu metodyki badań, analizie uzyskanych danych i recenzji finalnej wersji manuskryptu.

Wyrażam zgodę na włączenie w/w publikacji do postępowania habilitacyjnego dr Anny Puścion-Jakubik.



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mój udział polegał na pomocy w przeprowadzaniu oznaczeń, w tym na przygotowaniu niezbędnych odczynników.

Wyrażam zgodę na włączenie w/w publikacji do postępowania habilitacyjnego dr Anny Puścion-Jakubik.

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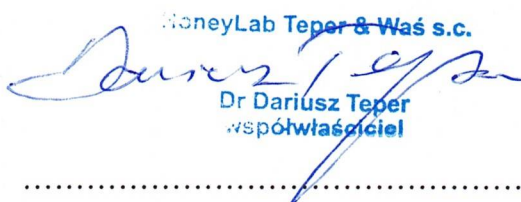
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mój udział polegał przeprowadzeniu analizy melissopalinologicznej miodów pochodzących z Włoch i Hiszpanii, analizie uzyskanych rezultatów oraz na korekcie finalnej wersji manuskryptu.

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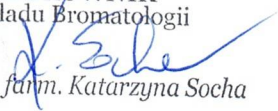
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mój udział polegał na recenzji finalnej wersji manuskryptu i sprawowaniu opieki merytorycznej.

Wyrażam zgodę na włączenie w/w publikacji do postępowania habilitacyjnego dr Anny Puścion-Jakubik.

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Oświadczenia współautorów publikacji

H4

Białystok, 19.12.2022 r.

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Szkoła doktorska

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OŚWIADCZENIE WSPÓŁAUTORA PUBLIKACJI

Oświadczam, że w publikacji:

Puścion-Jakubik A., Olechno E., Socha K., Zujko M.E. *Eating habits during the COVID-19 pandemic and the level of antibodies IgG and FRAP – experiences of Polish school staff: a pilot study*. Foods, 2022, 11(3), ID 408.

mój udział polegał na analizie uzyskanych danych, w tym prezentacji graficznej, jak również obsłudze specjalistycznego oprogramowania.

Wyrażam zgodę na włączenie w/w publikacji do postępowania habilitacyjnego dr Anny Puścion-Jakubik.

.....Ewa Olechno.....

(podpis współautora)

Białystok, 19.12.2022 r.

Dr hab. Katarzyna Socha
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Ul. Mickiewicza 2d
15-222 Białystok

OŚWIADCZENIE WSPÓŁAUTORA PUBLIKACJI

Oświadczam, że w publikacji:

Puścion-Jakubik A., Olechno E., Socha K., Zujko M.E. *Eating habits during the COVID-19 pandemic and the level of antibodies IgG and FRAP – experiences of Polish school staff: a pilot study*. Foods, 2022, 11(3), ID 408

mój udział polegał na analizie formalnej, recenzji finalnej wersji manuskryptu, zarządzaniu projektem, pozyskaniu finansowania i sprawowaniu opieki merytorycznej.

Wyrażam zgodę na włączenie w/w publikacji do postępowania habilitacyjnego dr Anny Puścion-Jakubik.

KIEROWNIK
Zakładu Bromatologii


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Białystok, 19.12.2022 r.

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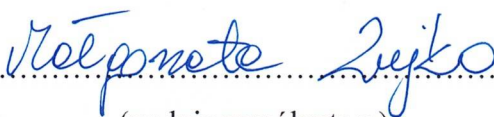
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Oświadczam, że w publikacji:

Puścion-Jakubik A., Olechno E., Socha K., Zujko M.E. *Eating habits during the COVID-19 pandemic and the level of antibodies IgG and FRAP – experiences of Polish school staff: a pilot study*. Foods, 2022, 11(3), ID 408.

mój udział polegał na stworzeniu koncepcji publikacji, opracowaniu metodologii badania, walidacji, dokonaniu analizy formalnej, recenzji finalnej wersji manuskryptu, sprawowaniu opieki merytorycznej i zarządzaniu projektem.

Wyrażam zgodę na włączenie w/w publikacji do postępowania habilitacyjnego dr Anny Puścion-Jakubik.

.....

(podpis współautora)

Oświadczenia współautorów publikacji

H5

Białystok, 19.12.2022 r.

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OŚWIADCZENIE WSPÓŁAUTORA PUBLIKACJI

Oświadczam, że w publikacji:

Puścion-Jakubik A., Markiewicz-Żukowska R., Naliwajko S.K., Gromkowska-Kępka K.J., Moskwa J., Grabia M., Mielech A., Bielecka J., Karpińska E., Mielcarek K., Nowakowski P., Socha K. *Intake of antioxidant vitamins and minerals in relation to body composition, skin hydration and lubrication in young women*. Antioxidants, 2021, 10(7), ID 1110

mój udział polegał na pomocy w opracowaniu koncepcji badań, przeprowadzeniu oznaczeń składu ciała, w tym na obsłudze specjalistycznego oprogramowania, walidacji, analizie formalnej, recenzji finalnej wersji manuskryptu, pomocy w zarządzaniu projektem i pozyskaniu finansowania.

Wyrażam zgodę na włączenie w/w publikacji do postępowania habilitacyjnego dr Anny Puścion-Jakubik.



(podpis współautora)

Białystok, 19.12.2022 r.

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OŚWIADCZENIE WSPÓŁAUTORA PUBLIKACJI

Oświadczam, że w publikacji:

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mój udział polegał na współtworzeniu koncepcji pracy, pomocy w opracowaniu metodyki badań, wykonywaniu analizy składu ciała, w tym na walidacji i obsłudze specjalistycznego oprogramowania, przeprowadzeniu analizy formalnej, pomocy w zarządzaniu projektem, w analizie danych i na recenzji finalnej wersji manuskryptu.

Wyrażam zgodę na włączenie w/w publikacji do postępowania habilitacyjnego dr Anny Puścion-Jakubik.


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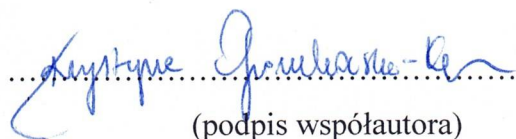
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mój udział polegał na współprowadzeniu badań (wykonywaniu analizy składu ciała, badaniu nawilżenia skóry i natłuszczenia skóry) oraz opracowywaniu danych.

Wyrażam zgodę na włączenie w/w publikacji do postępowania habilitacyjnego dr Anny Puścion-Jakubik.

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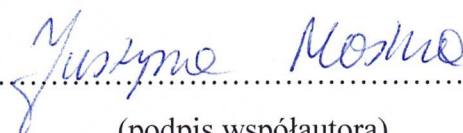
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mój udział polegał na pomocy w wykonywaniu analiz składu ciała, nawilżenia i natłuszczenia skóry, w tym obsłudze specjalistycznego oprogramowania.

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mój udział polegał na współprowadzeniu badań: wykonaniu analiz składu ciała i interpretacji wywiadów żywieniowych, w tym obsłudze specjalistycznego oprogramowania.

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mój udział polegał na łączeniu danych uzyskanych z różnych analiz, w tym na obsłudze specjalistycznego oprogramowania.

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mój udział polegał na przeprowadzaniu analizy składu ciała, w tym obsłudze specjalistycznego oprogramowania.

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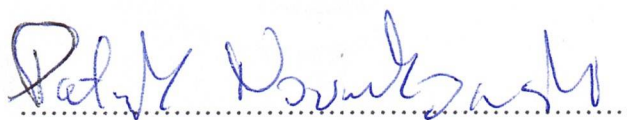
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mój udział polegał na współtworzeniu koncepcji publikacji, opracowaniu metodologii badań, walidacji, analizie formalnej, zarządzaniu projektem, pozyskaniu finansowania, recenzji finalnej wersji manuskryptu i sprawowaniu opieki merytorycznej.

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Oświadczenia współautorów publikacji

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†contributed equally

mój udział polegał na pomocy w opracowaniu metodologii badań, analizie uzyskanych danych ankietowych, w tym obsłudze specjalistycznego oprogramowania i przedstawieniu graficznym, pisaniu manuskryptu i wykonywaniu czynności administracyjnych w ramach projektu.

Wyrażam zgodę na włączenie w/w publikacji do postępowania habilitacyjnego dr Anny Puścion-Jakubik.

.....*Joanna Mielech*.....
(podpis współautora)

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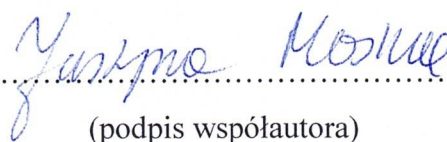
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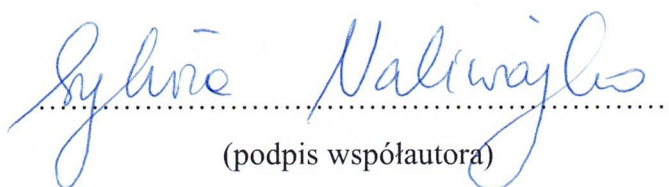
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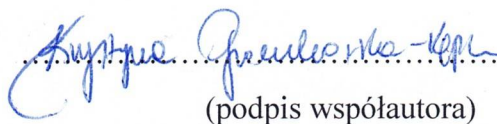
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†contributed equally

mój udział polegał na zbieraniu i opracowywaniu danych ankietowych.

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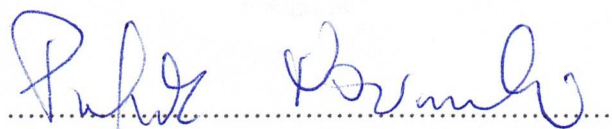
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Puścion-Jakubik A. †, Bielecka J. †, Grabia M., Mielech A., Markiewicz-Żukowska R., Mielcarek K., Moskwa J., Naliwajko S., Soroczyńska J., Gromkowska-Kępka K., Nowakowski P., Socha K. *Consumption of food supplements during the three COVID-19 waves in Poland – focus on zinc and vitamin D*. Nutrients, 2021, 13(10), ID 3361.

†contributed equally

mój udział polegał na walidacji, analizie formalnej, zarządzaniu projektem, recenzji finalnej wersji manuskryptu i sprawowaniu opieki merytorycznej.

Wyrażam zgodę na włączenie w/w publikacji do postępowania habilitacyjnego dr Anny Puścion-Jakubik.

KIEROWNIK
Zakładu Bromatologii

dr hab. n. farm. Katarzyna Socha

.....
(podpis współautora)

Oświadczenia współautorów publikacji

H7

Białystok, 19.12.2022 r.

Dr hab. Krzysztof Kus

Katedra i Zakład Farmakoeconomiki i Farmacji Społecznej

Uniwersytet Medyczny im. Karola Marcinkowskiego w Poznaniu

Ul. Rokietnicka 7

60-806 Poznań

OŚWIADCZENIE WSPÓŁAUTORA PUBLIKACJI

Oświadczam, że w publikacji:

Puścion-Jakubik A., Kus K., Socha K. *Medical university students' perspective on marketing of dietary supplements*. Acta Poloniae Pharmaceutica, 2021, 78(2), 205-218
mój udział polegał pomocy w formułowaniu wniosków oraz na korekcie finalnej wersji manuskryptu.

Wyrażam zgodę na włączenie w/w publikacji do postępowania habilitacyjnego dr Anny Puścion-Jakubik.

dr hab. n. farm.
Krzysztof Kus

.....
(podpis współautora)

Białystok, 19.12.2022 r.

Dr hab. Katarzyna Socha
Kierownik Zakładu Bromatologii
Uniwersytetu Medycznego w Białymstoku
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15-222 Białystok

OŚWIADCZENIE WSPÓŁAUTORA PUBLIKACJI

Oświadczam, że w publikacji:

Puścion-Jakubik A., Kus K., Socha K. *Medical university students' perspective on marketing of dietary supplements*. Acta Poloniae Pharmaceutica, 2021, 78(2), 205-218
mój udział polegał na recenzji finalnej wersji manuskryptu i sprawowaniu opieki merytorycznej.

Wyrażam zgodę na włączenie w/w publikacji do postępowania habilitacyjnego dr Anny Puścion-Jakubik.

KIEROWNIK
Zakładu Bromatologii


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Oświadczenia współautorów publikacji

H8

Białystok, 19.12.2022 r.

Mgr Natalia Bartosiewicz

Absolwentka Uniwersytetu Medycznego w Białymstoku

Kierunek: Farmacja

OŚWIADCZENIE WSPÓŁAUTORA PUBLIKACJI

Oświadczam, że w publikacji:

Puścion-Jakubik A. †, Bartosiewicz N. †, Socha K. *Is the magnesium content in food supplements consistent with the manufacturers' declaration?* Nutrients, 2021, 13(10), ID 3416. †**contributed equally**

mój udział polegał na pomocy w wykonywaniu mineralizacji i oznaczenia zawartości magnezu w suplementach diety, analizie uzyskanych rezultatów i przedstawieniu ich w sposób graficzny, w tym na obsłudze specjalistycznego oprogramowania, oraz na pisaniu części manuskryptu.

Wyrażam zgodę na włączenie w/w publikacji do postępowania habilitacyjnego dr Anny Puścion-Jakubik.

Natalia Bartosiewicz

(podpis współautora)

Białystok, 19.12.2022 r.

Dr hab. Katarzyna Socha
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Uniwersytetu Medycznego w Białymstoku
Ul. Mickiewicza 2d
15-222 Białystok

OŚWIADCZENIE WSPÓLAUTORA PUBLIKACJI

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Puścion-Jakubik A. †, Bartosiewicz N. †, Socha K. *Is the magnesium content in food supplements consistent with the manufacturers' declaration?* Nutrients, 2021, 13(10), ID 3416. †**contributed equally**

mój udział polegał na pomocy merytorycznej w przeprowadzeniu oznaczenia zawartości magnezu w suplementach diety, w tym obsłudze specjalistycznego oprogramowania, walidacji, recenzji finalnej wersji manuskryptu i sprawowaniu opieki merytorycznej.

Wyrażam zgodę na włączenie w/w publikacji do postępowania habilitacyjnego dr Anny Puścion-Jakubik.

KIEROWNIK
Zakładu Bromatologii

dr hab. n. farm. Katarzyna Socha

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(podpis współautora)

Oświadczenia współautorów publikacji

H9

Białystok, 19.12.2022 r.

Mgr Gabriela Staniaszek

Studenckie Koło Naukowe przy Zakładzie Bromatologii

Uniwersytetu Medycznego w Białymstoku

Kierunek: Farmacja

OŚWIADCZENIE WSPÓŁAUTORA PUBLIKACJI

Oświadczam, że w publikacji:

Puścion-Jakubik A. †, Staniaszek G. †, Brzozowska P., Socha K. *Quality of calcium food supplements: evaluation compared to manufacturers' declarations*. Molecules, 2022, 27(23), ID 8154.

†contributed equally

mój udział polegał na pomocy w analizie danych i przedstawieniu ich w formie graficznej, w tym obsłudze specjalistycznego oprogramowania, walidacji i pisaniu manuskryptu.

Wyrażam zgodę na włączenie w/w publikacji do postępowania habilitacyjnego dr Anny Puścion-Jakubik.

Gabriela Staniaszek

(podpis współautora)

Białystok, 19.12.2022 r.

Mgr Patrycja Brzozowska

Studenckie Koło Naukowe przy Zakładzie Bromatologii

Uniwersytetu Medycznego w Białymstoku

Kierunek: Farmacja

OŚWIADCZENIE WSPÓŁAUTORA PUBLIKACJI

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Wyrażam zgodę na włączenie w/w publikacji do postępowania habilitacyjnego dr Anny Puścion-Jakubik.

Patrycja Brzozowska.....
(podpis współautora)

Białystok, 19.12.2022 r.

Dr hab. Katarzyna Socha
Kierownik Zakładu Bromatologii
Uniwersytetu Medycznego w Białymstoku
Ul. Mickiewicza 2d
15-222 Białystok

OŚWIADCZENIE WSPÓŁAUTORA PUBLIKACJI

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mój udział polegał na współtworzeniu koncepcji publikacji, pomocy merytorycznej w przeprowadzeniu oznaczenia zawartości wapnia w suplementach diety, analizie formalnej, zarządzaniu projektem, pozyskaniu finansowania, recenzji finalnej wersji manuskryptu i sprawowaniu opieki merytorycznej.

Wyrażam zgodę na włączenie w/w publikacji do postępowania habilitacyjnego dr Anny Puścion-Jakubik.

KIEROWNIK
Zakładu Bromatologii


dr hab. n. farm. Katarzyna Socha

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(podpis współautora)

Oświadczenia współautorów publikacji

H10

Białystok, 19.12.2022 r.

Mgr Anita Żmudzińska

(nazwisko panięskie: Mielech)

Szkoła doktorska

Zakład Bromatologii

Uniwersytetu Medycznego w Białymstoku

Ul. Mickiewicza 2d

15-222 Białystok

OŚWIADCZENIE WSPÓŁAUTORA PUBLIKACJI

Oświadczam, że w publikacji:

Puścion-Jakubik A., Mielech A., Abramiuk D., Iwaniuk M., Grabia M., Bielecka J., Markiewicz-Żukowska R., Socha K. *Mercury content in dietary supplements from Poland containing ingredients of plant origin: a safety assessment*. *Frontiers in Pharmacology*, 2021, 12, ID 738549

mój udział polegał na pomocy w oznaczaniu zawartości rtęci w badanych suplementach diety i analizie uzyskanych wyników.

Wyrażam zgodę na w/w publikacji do postępowania habilitacyjnego dr Anny Puścion-Jakubik.

Żmudzińska Anita

(podpis współautora)

Białystok, 19.12.2022 r.

Mgr Dominika Abramiuk
Studenckie Koło Naukowe przy Zakładzie Bromatologii
Uniwersytetu Medycznego w Białymstoku
Kierunek: Farmacja

OŚWIADCZENIE WSPÓŁAUTORA PUBLIKACJI

Oświadczam, że w publikacji:

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mój udział polegał na pomocy w przeprowadzeniu oznaczeń zawartości rtęci w suplementach diety, w tym obsłudze specjalistycznego oprogramowania, jak również na analizie uzyskanych danych.

Wyrażam zgodę na włączenie w/w publikacji do postępowania habilitacyjnego dr Anny Puścion-Jakubik.

Abramiuk Dominika

(podpis współautora)

Białystok, 19.12.2022 r.

Mgr Małgorzata Iwaniuk

Studenckie Koło Naukowe przy Zakładzie Bromatologii

Uniwersytetu Medycznego w Białymstoku

Kierunek: Farmacja

OŚWIADCZENIE WSPÓŁAUTORA PUBLIKACJI

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.....Małgorzata Iwaniuk.....
(podpis współautora)

Białystok, 19.12.2022 r.

Mgr Monika Grabia
Zakład Bromatologii
Uniwersytetu Medycznego w Białymstoku
Ul. Mickiewicza 2d
15-222 Białystok


OŚWIADCZENIE WSPÓŁAUTORA PUBLIKACJI

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mój udział polegał na współprowadzeniu badań (oznaczaniu zawartości rtęci w suplementach diety), analizie uzyskanych danych, w tym obsłudze specjalistycznego oprogramowania oraz pisaniu manuskryptu.

Wyrażam zgodę na włączenie w/w publikacji do postępowania habilitacyjnego dr Anny Puścion-Jakubik.

..........
(podpis współautora)

Białystok, 19.12.2022 r.

Mgr Joanna Bielecka
Zakład Bromatologii
Uniwersytetu Medycznego w Białymstoku
Ul. Mickiewicza 2d
15-222 Białystok

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.....
Joanna Mielech
(podpis współautora)

Białystok, 19.12.2022 r.

Dr hab. Renata Markiewicz-Żukowska
Zakład Bromatologii
Uniwersytetu Medycznego w Białymstoku
Ul. Mickiewicza 2d
15-222 Białystok

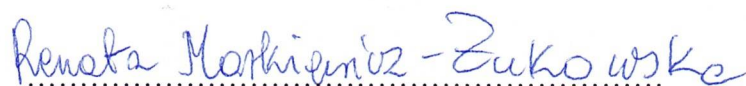
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mój udział polegał na współtworzeniu koncepcji manuskryptu, przeprowadzeniu analizy formalnej i recenzji finalnej wersji manuskryptu.

Wyrażam zgodę na włączenie w/w publikacji do postępowania habilitacyjnego dr Anny Puścion-Jakubik.



(podpis współautora)

Białystok, 19.12.2022 r.

Dr hab. Katarzyna Socha
Kierownik Zakładu Bromatologii
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KIEROWNIK
Zakładu Bromatologii


dr hab. n. farm. Katarzyna Socha

.....
(podpis współautora)