Quality assessment and dissolution properties of dietary supplements with isoflavones

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Summary
Determination of the detailed chemical composition of active compounds in dietary supplements and their release from dosage form is not obligatory, although these parameters are crucial for obtaining the desired health effect. Six commercially available dietary supplements with soya (four products) and red clover (two products) extract, used for alleviation of menopausal symptoms, were chemically characterized and several tests of solid dosage forms were performed. Quantification of isoflavones was conducted using high-performance liquid chromatography. In three supplements total isoflavone content varied by ± 10 % from the declared content, while in other samples the values ranged from + 14.6 % to + 63.4 %. Daidzein and its conjugates were the most abundant isoflavones in all soybean-based dietary supplements, while formononetin was dominant in red clover supplements. Three products did not have desirable uniformity of dosage units. Dissolution test showed that only two soy-based supplements released more than 75 % of the declared isoflavone content in 45 min. Isoflavones from both red clover products were poorly (less than 5 %) dissolved. The obtained results indicate that more rigorous control of the production process and of the final products is needed in order to provide high quality dietary supplements.

Keywords
dietary supplement; quality control; isoflavone; dissolution test

Global climate change, pandemic and war crisis induced significant impact on food sector. Reduced crop productivity, disruption of supply chains, generally diminished food safety and quality are challenges currently present in food industry. Crisis also emphasized the need for innovations and sustainability in food production. Additionally, COVID-19 pandemic increased promotion and interest in high quality nutraceuticals and supplements with high concentration of natural bioactive ingredients in the general population [1, 2].

Constant rise of the consumption of dietary supplements in Europe is noticeable and estimation is that approximately 20 % of Europeans use at least one food supplement [3]. Nowadays, internet marketing and on-line availability of these products makes them even more attractive for consumers. Preparations containing soya are among 10 most frequently used herbal supplements [4]. These products, rich in phytoestrogen compounds, are mainly recommended for treatment of unpleasant menopausal symptoms such as hot flushes, insomnia and mood changes. They can also be beneficial in prevention of osteoporosis and cardiovascular diseases, along with antioxidant and immune-boosting activities. Although hormone replacement therapy is usually prescribed during menopause, it is often avoided by women due to the fear of severe adverse effects (i.e. breast cancer, heart attack), as well as misunderstanding of the risk/benefit ratio. This fact also contributes to the popularity of dietary supplements containing phytoestrogens. Due to their similarity to 17-β-estradiol, these natural compounds can bind to estrogen receptors and modify hormone signaling. On the other hand, they can also exert various estrogen-independent effects [5].

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One of the major classes of phytoestrogens are isoflavones, secondary metabolites present in different plant species of the Fabaceae family. Soya (*Glycine max* L.) and red clover (*Trifolium pratense* L.) are dominant sources of isoflavones, mainly used as raw materials for production of supplements used in menopause [6]. Soybean contains isoflavone aglycones (daidzein, genistein, glycitein) and their β-glucoside, acetyl glucoside and malonyl glucoside forms [7]. Red clover may also contain daidzein and genistein, but it is usually rich in their methylated derivatives, namely, formononetin and biochanin A, along with their corresponding glucosides [8]. Recent research indicated that various by-products obtained from food production (e.g. soya molasses, soya curd, oat and wheat bran) could also be considered as valuable sources of isoflavones and other antioxidant compounds [9]. Application of innovative approaches for recovery of antioxidants to this type of waste materials could be useful for obtaining sustainable products in the food and pharmaceutical sectors.

Bearing in mind the diversity of isoflavones present in these sources, detailed chemical profiling of supplements with these bioactive components is desirable. However, requirements concerning dietary supplements in European countries are not harmonized and generally these products are not subjected to strict quality control by standardized procedures. It is expected that producers apply some quality management system (for example Good Manufacturing Practice) in order to obtain safe products. Nevertheless, the manufacturer's label usually provides only limited information about product composition [10]. Consequently, consumers and health professionals are not able to estimate credibility of available efficacy claims. Previous research studies on vitamin, mineral and herbal dietary supplements showed that herbal products are particularly prone to variation due to great differences in the cultivation, environmental and storage conditions of plant materials [11]. Also, due to the possible presence of various contaminants (heavy metals, pesticides, mycotoxins), these products demand strict safety control [12].

Pharmaceutical testing of finished dosage forms (uniformity of dosage units, dissolution testing) is an important aspect in estimation of product quality. Dissolution phase is often the critical point that determines absorption rate of active compounds from solid dosage forms for oral application. Testing of dissolution properties can be used for bioavailability assessment, which is crucial for obtaining the desired pharmacological effect. Of course, there are many factors influencing dissolution profile of specific compounds such as solubility, particle size, additional ingredients and disintegration rate of dosage form. Therefore, the aim of this study was to perform dissolution test in order to assess the amount of total and individual isoflavones released from solid dosage forms of commercially available dietary supplements based on soya and red clover. Isoflavone profile was also determined and the content of active compounds was compared with the values stated on the label. Additionally, uniformity of dosage units was examined. This approach could provide better insight in the current state on the market concerning quality of dietary supplements and reveal the potential shortcomings of this type of products.

MATERIALS AND METHODS

Samples and chemicals

Six commercially available dietary supplements (4 soya-based and 2 red clover-based) from Serbian pharmacies were tested. Detailed product information is given in Tab. 1. Standards of daidzein, glycitein and genistein were obtained from ChromaDex (Irvine, California, USA) and of formononetin and biochanin A were obtained from Sigma Aldrich (St. Louis, Missouri, USA). Acetic acid (min. 99.5 %) and HCl (35 %) were purchased from POCH (Gliwice, Poland). Sodium acetate trihydrate (99 %) was obtained from Lach-ner (Neratovice, Czech Republic). Sulfuric acid (p. a.) was obtained from RTB (Bor, Serbia). Sodium phosphate monobasic and dibasic were obtained from Sigma Aldrich. Methanol and acetonitrile were of high-performance liquid chromatography (HPLC) grade and were obtained from J. T. Baker (Deventher, the Netherlands).

Uniformity of mass and uniformity of dosage units

Uniformity of mass of single dose preparations and uniformity of dosage units (mass variation test) for hard capsules and uncoated tablets was determined according to general monographs (2.9.5. and 2.9.40.) of European Pharmacopoeia 11th edition [13].

Sample preparation

Content of 10 tablets or capsules was pulverized and mixed, and analysis (in triplicate) was performed on amount of sample equal to average mass of dosage unit, in order to determine the average content of isoflavones. Isoflavone extraction was conducted with 80 % methanol during 2 h at 40 °C with constant stirring.

Tab. 1. Information on dietary supplements declared on the label.

Sample	Dosage form	Declared ingredients	Recommended daily dose	Isoflavones * [mg]
S1	Hard capsules	Standardized soya isoflavone extract with 40 % of isoflavones (100 mg); magnesium stearate, microcrystalline cellulose	1 capsule	40
S2	Hard capsules with prolonged release	Standardized soya isoflavone extract with 30 % of isoflavones (133 mg); lactose monohydrate, talcum, gelatin	1 capsule	40
S3	Tablets	Soya concentrate (700 mg), standardized root extract of *Cimicifuga racemosa* (53.34 mg, 2.5 % triterpene glycoside), root extract of *Angelica sinensis (*50 mg), root extract of *Glycyrrhiza glabra (*50 mg) and fruit extract of *Vitex agnus-castus (*33.34 mg)	1 tablet	21.67
S4	Hard capsules	Soya concentrate (62.5 mg), dicalcium phosphate, gelatin, vitamin C, vitamin E, zinc oxide, silicic acid, titanium dioxide, folic acid, biotin	2 capsules	25
S5	Hard capsules	Standardized dry red clover extract with 40 % of isoflavones (100 mg), lactose, gelatin, microcrystalline cellulose, talcum, magnesium stearate, silicon dioxide, colours	1 capsule	40
S6	Hard capsules	Standardized dry red clover extract with 40 % of isoflavones (100 mg), microcrystalline cellulose, silicon dioxide, magnesium stearate, gelatin, titanium dioxide, colours	1 capsule	40

* – content per capsule or tablet.

HPLC for isoflavone analysis

A model 1100 series HPLC (Agilent Technologies, Santa Clara, California, USA) equipped with a binary pump, degasser, auto sampler and diode array detector (DAD) was used to separate, identify, and quantify isoflavones. The analysis of soya isoflavones was conducted according to the method described by Lee et al. [7]. Separation of compounds was achieved using a Zorbax SB C18 reversed-phase HPLC column (4.6 mm × 150 mm, particle size 5 μm; Agilent Technologies) with a Zorbax SB C18 guard column (12.5 mm × 4.6 mm, particle size 5 μm; Agilent Technologies) at temperature 25 °C, mobile phase flow 0.6 ml·min^{-1}, injection volume 10 μl and detection wavelength 270 nm. Mobile phase gradient was formed with solvent A (1 % v/v acetic acid in water), and solvent B (100 % acetonitrile). Isoflavones were identified by comparing the retention times and UV spectra of samples with those of standards and literature data [7]. Five-point regression curves ($r \geq 0.9998$) of daidzein, glycitein and genistein standard compounds were used. For quantification of glucoside forms, calibration curves of corresponding aglycone compounds were used and corrections for differences in molecular weight between aglycones and glucosides were calculated according to the formula:

$$c_x = c_y \times \frac{M_x}{M_y} \quad (1)$$

where c_x is concentration of glucoside (in milligrams per millilitre), c_y is concentration of aglycone (in milligrams per millilitre), M_x is molecular weight of glucoside and M_y is molecular weight of aglycone.

The total content of isoflavones was expressed in aglycone equivalents by summing the masses of the corresponding components without applying the correction for differences in molecular weight between aglycones and glucosides.

HPLC conditions for analysis of red clover-based dietary supplements were according to the validated method of Krenn et al. [14]. Separation of isoflavones from red clover-based dietary supplements was achieved using a Zorbax SB C18 column (4.6 mm × 250 mm, particle size 5 μm; Agilent Technologies) at temperature 25 °C, mobile phase flow 1 ml·min^{-1}, injection volume 10 μl and detection wavelength 254 nm. Mobile phase gradient was formed with solvent A (water of pH 2.7, adjusted with sulfuric acid) and solvent B (acetonitrile). Five-point calibration curves ($r \geq 0.999$) of daidzein, genistein, formononetin and biochanin A standard compounds were used for quantification.

Samples were filtered through regenerated cellulose filters, pore size 0.45 μm (Agilent Technologies) prior to HPLC analysis.

Dissolution test

A dissolution tester, type DT 800 (Erweka, Langen, Germany) was used to determine the dissolution rate of isoflavones. This testing on

6 dosage units of each dietary supplement was in accordance with European Pharmacopoeia [13] requirements in a general monograph for Dissolution test for solid dosage forms (2.9.3.). Apparatus 1 (basket) was applied with acetate buffer pH 4.5 (500–900 ml). Additionally, media at pH 1.2 (0.1 mol·l^{-1} HCl) or pH 6.8 (phosphate buffer) were used for samples which did not dissolve in acetate buffer. Applied speed was 1.67 Hz. Sampling for conventional-release dosage forms was performed at 15, 30, 45, 60, 90, 120 and 150 min, while for the sample with prolonged release (S2), the test time points were 30, 60, 120, 180, 300, 720 and 1 440 min. The obtained samples were analysed by the appropriate previously described HPLC method to determine isoflavones.

RESULTS AND DISCUSSION

Uniformity of mass and uniformity of dosage units

European Pharmacopoeia requirements [13] for uniformity of mass were met for all analysed samples. Criteria for uniformity of dosage units were met only by samples S4, S5 and S6. Supplements S1, S2 and S3 failed to comply with the performed test because of the high variations of total isoflavone content among individual dosage units (from 20 % to 60 % of average content). These variations in the content of active compounds in dosage units of individual batches could be the result of inadequate homogenization of the extract during production. The lack of uniformity of dosage units in this case would consequently lead to great differences in patient's daily isoflavone intake.

Isoflavones content in dietary supplements

Results obtained for the total isoflavone content are presented in Tab. 2. Samples S1, S5 and S6 had total isoflavone content in ±10% range from the declared value. The remaining three products contained higher levels of total isoflavones, ranging from + 14.6 % to + 63.4 % from the declared content. Manufacturers generally do not specify if the isoflavone content of the dietary supplement regards aglycone or glycoside form, which makes interpretation of the results difficult [15]. High discrepancies from the declared isoflavone content in this study (noticed for samples S2, S3 and S4) were obtained despite expressing the results as aglycone equivalents (Tab. 2). These differences would be even more pronounced if the results were presented as glycosides. Several previously performed studies also reported that the content of isoflavones in products differed from the declared value [14, 15]. Results published by Mornar et al. [10] showed that the content of phytoestrogens in supplements was mostly below the content stated by the manufacturer, while in some cases these compounds were not even present in the analysed sample. The variability in the content of active compounds between dietary supplement batches was also identified as a major problem in herbal products [10]. On the other hand, in the study performed by Andres et al. [6] it was shown that total isoflavone content in dietary supplements based on soya and red clover was mainly in accordance with the label, but only when isoflavone content was expressed as its conjugated form.

Recommended daily intake, given on the label of tested dietary supplements, ranged from 21.67 mg to 50 mg isoflavones per day. Our experimental results showed that real daily intake of isoflavones would actually be from 31.22 mg to 81.72 mg. Significant differences among daily intake of isoflavones calculated after dietary supplement analysis were also noticed by other authors (from 5 mg to 186 mg) showing that consumers may receive misleading information about their daily intake [16]. Rizzo and Baroni [17] stated

Tab. 2. Total isoflavones per average mass of capsule or tablet and their deviation from the declared content.

Isoflavones	S1	S2	S3	S4	S5	S6
TI determined [mg]	40.10	45.86	31.22	40.86	40.55	37.42
Deviation from declared TI content [%]	+ 0.3	+ 14.6	+ 44.1	+ 63.4	+ 1.4	− 6.5
Total daidzein [mg]	25.59	25.59	21.92	30.71	15.51	0.23
Total glycitein [mg]	3.83	1.69	7.58	10.15	NA	NA
Total genistein [mg]	10.67	18.57	1.71	–	7.42	0.24
Formononetin [mg]	NA	NA	NA	NA	17.07	19.36
Biochanin A [mg]	NA	NA	NA	NA	0.56	17.71

TI – total isoflavones (sum of total daidzein, glycitein, genistein, formononetin and biochanin), NA – not analysed.
S1, S2, S3, S4, S5, S6 – specification of samples is given in Tab. 1.

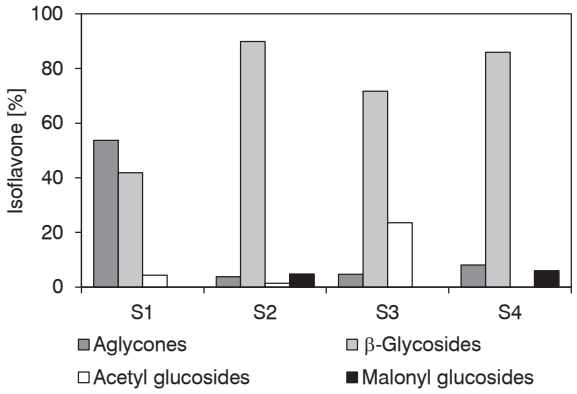

Fig. 1. Contribution of isoflavone forms in soya-based dietary supplements.

S1, S2, S3, S4 – specification of samples is given in Tab. 1.

Tab. 3. Content of individual isoflavones per average mass of capsule or tablet in soya-based dietary supplements.

Isoflavones	S1	S2	S3	S4
Daidzein [mg]	13.11	1.15	NQ	2.53
Daidzin [mg]	18.19	36.71	26.25	42.16
Acetyl daidzin [mg]	2.47	1.14	10.64	NQ
Malonyl daidzin [mg]	NQ	2.76	NQ	4.81
Glycitein [mg]	0.96	0.33	1.48	0.78
Glycitin [mg]	3.92	2.13	7.31	14.72
Acetyl glycitin [mg]	0.65	NQ	2.48	NQ
Genistein [mg]	7.47	0.29	NQ	NQ
Genistin [mg]	5.12	27.94	2.73	NQ
Malonyl genistin [mg]	NQ	1.58	NQ	NQ

S1, S2, S3, S4 – specification of samples is given in Tab. 1.
NQ – not quantified.

that generally recommended isoflavone intake should be from 60 mg to 100 mg per day in order to obtain beneficial health effects. WATANABE and UEHARA [5] also concluded that recommended isoflavone dosage might be around 100 mg per day. These values correspond to those observed in countries where high amounts of soya are consumed, e.g. Japan and China. Therefore, it would be useful to harmonize isoflavones content in dietary supplements with current scientific data, since the determined values in all analysed samples were mostly below these recommendations. At the same time, isoflavones intake in Europe is continuously increasing due to the high consumption of soya products by vegetarians and vegans. Therefore, in 2015 the European Food Safety Authority (EFSA) [18] conducted a systematic review of the available data associated with soya and red clover isoflavones intake in order to assess their safety. The focus was on mammary, uterus and thyroid organ effects. It was concluded that doses up to 150 mg of isoflavones per day during several months of consumption could be regarded as safe [18]. Isoflavones intake through the tested products could be considered safe, even with additional dietary consumption of soya foods.

Individual isoflavones content in soya-based dietary supplements

The performed analysis showed that daidzein and its conjugates were the most abundant in all four soybean-based dietary supplements (Tab. 2), comprising more than 50 % of the total isoflavones content (55.8 % to 75.2 %). The observed high percentage of glyciteins in S3 (24.3 %) and S4 (24.8 %) might be considered unusual, since these compounds are generally the least present in soya seed. Similar observation was made by FIECHTER et al. [19] where one of 11 analysed samples contained substantial amount of glyciteins (27.8 %), while in others daidzein or genistein were the dominant aglycones. Other authors noticed that genistein or daidzein are isoflavone types mainly present in dietary supplements, while the content of glycitein was significantly lower [16].

Results on glycosides showed that the most of the analysed dietary supplements contained β-glucosides as dominantly present (Fig. 1). Only in S1, aglycone forms prevailed over β-glucoside forms, accounting for 53.7 % of the total content of isoflavones. Other samples contained low percentage of aglycon forms. Malonyl forms were only found in samples S2 and S4 (Fig. 1). Although malonyl forms of isoflavones are often dominant in the raw soya seeds [20], the analysis showed that β-glucoside forms are most abundant in dietary supplements (Tab. 3). This could be due to treatment of extract with heat and/or in the presence of acid during the production, which leads to decarboxylation of the unstable malonyl residue or its hydrolysis to β-glucoside form. STÜRTZ et al. [15] showed that β-glucoside isoflavones (glycitin, genistin and daidzin) prevailed in all 11 analysed samples of soybean-based dietary supplements. This study did not confirm the presence of malonyl glucosides in dietary supplements. FIECHTER et al. [19] detected high amounts of malonyl conjugates only in 1 from 11 samples from Austria, indicating that this supplement was probably produced using mild processing conditions.

Concerning individual compounds, 10 out of 12 tested isoflavones were present in the analysed dietary supplements (Tab. 3). In all samples,

daidzin was present at highest contents, while the content of other compounds varied. For example, sample S2 had notably high content of genistin, while in sample S1 high contents of daidzein could be observed (Tab. 3). Similar to our study, ANDRES et al. [6] found that daidzin was the dominant compound in 4 from 6 analysed soya-based dietary supplements.

Generally, isoflavones profile in soya-based supplements is similar to that of raw soya seed with daidzeins or genisteins being dominant compounds, while glyciteins are the least present [20]. The soya germ is richer in daidzin and glycitin, while genistin is mainly found in cotyledons [21]. Thus, presence of an extract rich in soya germs could be the reason for the high content of glyciteins in S3 and S4 (Tab. 2).

It was shown that from all soya isoflavones, genistein has the highest affinity to the estrogen receptors (approximately 10–20 times more than daidzein) and accordingly the most pronounced estrogenic effect. The binding affinity of daidzein is slightly lower while that of glycitein is the lowest [5]. Therefore, the dietary supplement containing the highest content of genistein and its conjugates (S2) could have the most favourable composition of isoflavones from the aspect of biological effects. Nevertheless, it is known that daidzein is metabolized to equol, which is the most active of all isoflavone metabolites, so this also should be taken into account for assessment of optimal isoflavone content.

Individual isoflavones content in red clover-based dietary supplements

Red clover-based dietary supplements contained all four analysed isoflavones (daidzein, genistein, formononetin and biochanin A; Tab. 2). Formononetin was the dominant isoflavone in both samples (S5 with 42.1 % and in S6 with 51.7 %). Concerning the contents of other isoflavones, it was shown that isoflavone profile of S6 was analogical to the usual plant material composition, while high daidzein and low biochanin A were noticeable in sample S5 (Tab. 2).

Previously it formononetin was established as the major compound in most of red clover dietary supplements [6]. Our results obtained for samples S5 and S6 were in accordance with that (Tab. 2). HOWES et al. [22] found that commercial a red clover dietary supplement had high contents of biochanin A and formononetin, while contents of daidzein and genistein were low. It was also shown that biochanin A and formononetin are major compounds in red clover [8] and, therefore, the profile of isoflavones determined for S5 was unexpected (Tab. 2). It is known that high variability of isoflavones content in red clover dietary supplements has impact on isoflavones absorption and metabolism.

Dissolution test

General requirements given by European Pharmacopoeia [13] state that more than 75 % of the declared content should be released in 45 min for conventional-release forms. Requirements regarding prolonged-release dosage forms include analysis in at least three time points in order to prove the gradual release of the active substance. According to manufacturer declarations, only S2 was oral dosage form with prolonged release, while other supplements were considered as oral dosage forms with conventional release. The released amounts of total isoflavones compared to the declared content in soya-based dietary supplements are presented in Fig. 2. Only supplements S2 and S3 fulfilled the criteria of Pharmacopoeia. Sample S3 released 145–148 % of the declared content after 45 min (Fig. 2A). Since the determined total isoflavones content in S3 was 144 % of the declared content, this high percentage is not surprising. On the other hand, in samples S1 and S4 percentage of released isoflavones in 6 individually tested dosage units after 45 min varied from 50 % to 58 % and from 47 % to 101 %, respectively. After 60 min, it varied from 50 % to 58 % in S1 and from 66 % to 113 % in S4 (average values with standard deviations are presented in Fig. 2A). Supplement S4 did not meet the dissolution criteria, because the appropriate amount of isoflavones was not released from all 6 tested dosage units, as can be seen from high standard deviation obtained for this sample (Fig. 2A). Dissolution profile of soya-based supplement with prolonged release (S2) was gradual, as after 30 min more than 30 % of the content was dissolved and more than 50 % of isoflavones were released after 120 min. Finally, after 720 min more than 75 % of the total isoflavones content was dissolved (Fig. 2B).

On the other hand, samples with red clover extract (S5 and S6) practically did not dissolve in the medium used (acetate buffer pH 4.5). Visually, they were partially disintegrated. Even after 150 min, not more than 5 % of the declared content of isoflavones was dissolved from red clover dietary supplements. For these samples, dissolution tests were carried out also at pH 1.2 and pH 6.8, but the dissolution rate did not exceed 10 % of the declared content (data not shown). This result could be a consequence of gelatin cross-linking in the tested red clover capsules, but

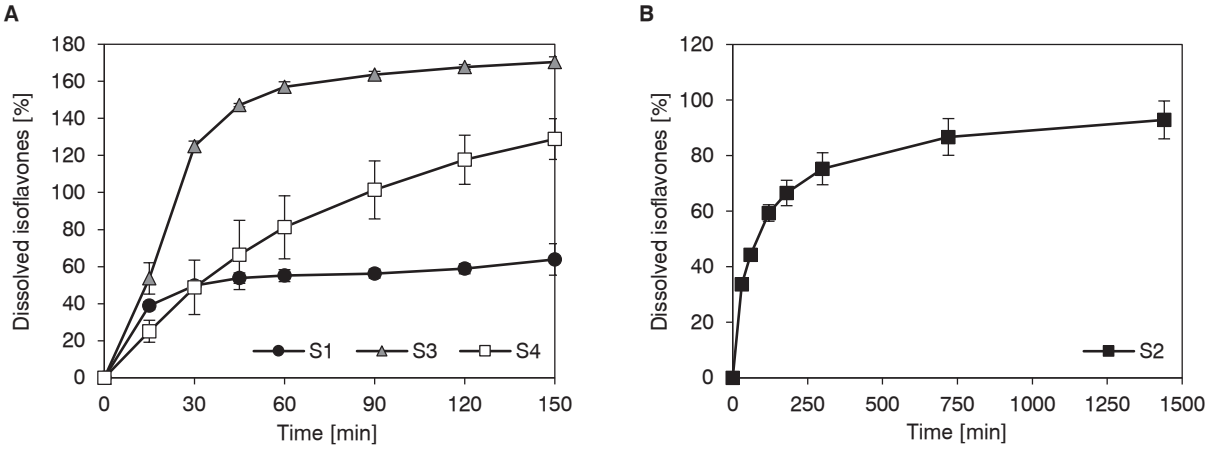

Fig. 2. Comparison of dissolution profiles of isoflavones in soya-based dietary supplements.

A – samples with conventional release, B – sample with prolonged release.
Average values for 6 dosage units with standard deviation is presented. S1, S2, S3, S4 – specification of samples is given in Tab. 1.

it is difficult to establish the real reason for this phenomenon.

Dissolution profiles of individual isoflavone forms in soya-based dietary supplements are presented in Fig. 3. Daidzin was the dominantly released compound from all samples, which was expected since this isoflavone is the main component of the tested dietary supplements. In samples with conventional release, it could be observed that daidzin reached its maximum release after 60 min in S1 and S3, while in sample S4 it was not reached even after 150 min. It could be also noticed that average contribution of these compounds to total isoflavones in the formulation did not correspond with their share in the final solutions at the end point of dissolution testing (Tab. 4). The greatest difference was observed in S1, where genistein accounted for 18.6 % of total isoflavones content in formulation. After capsule dissolution, genistein contributed to the final solution with only 3.1 % of total isoflavones. Also, daidzein accounted for 32.7 % of total isoflavones content in formulation, while this compound contributed only with 9.0 % to the final solution (Tab. 4).

To the best of our knowledge, there is currently no study available reporting dissolution profiles of commercial dietary supplements containing isoflavones, although this test is especially important for poorly water-soluble compounds, with low membrane permeability, such as isoflavones [23]. Poor dissolution properties of most of the tested supplements (S1, S4, S5, S6) indicated that probably the formulations are not adequate and that, consequently, bioavailability and efficiency of these products could be questionable. In this case, addition of various additives (surfactants, enzymes) to dissolution media could be considered. For example, for in-house genistein capsules the best dissolution properties were obtained in 3% sodium dodecyl sulphate [24]. Also, bearing in mind complexity of isoflavones composition in dietary supplements (in this study, presence of ten compounds) it is recommended to define an appropriate analytical marker substance. For soya-based supplements, daidzin could be the potential candidate for this purpose, as it is shown in Fig. 3.

Some suggestions for improvement of dissolution properties of isoflavones were made by other authors. In a study published by DE OLIVEIRA et al. [25], five immediate release tablet formulations with dried soya extract were produced using various disintegration agents, surfactants and diluents. The formulation with sodium croscarmellose and sodium dodecyl sulfate showed the fastest genistein and daidzein release. In another experiment, slow-release formulation with soybean isoflavone extract was developed. Extract was compacted with microcrystalline cellulose, coated with a mixture of ethyl cellulose and hydroxypropyl cellulose, and filled in hard gelatin capsules. The procedure significantly increased daidzein dissolution rate, while this effect was not noticed for genistein [26]. MAMAGKAKI et al. [24] prepared five different genistein capsules in order to obtain a product with the best pharmacokinetic properties. Dissolution test was also included in that study as an im-

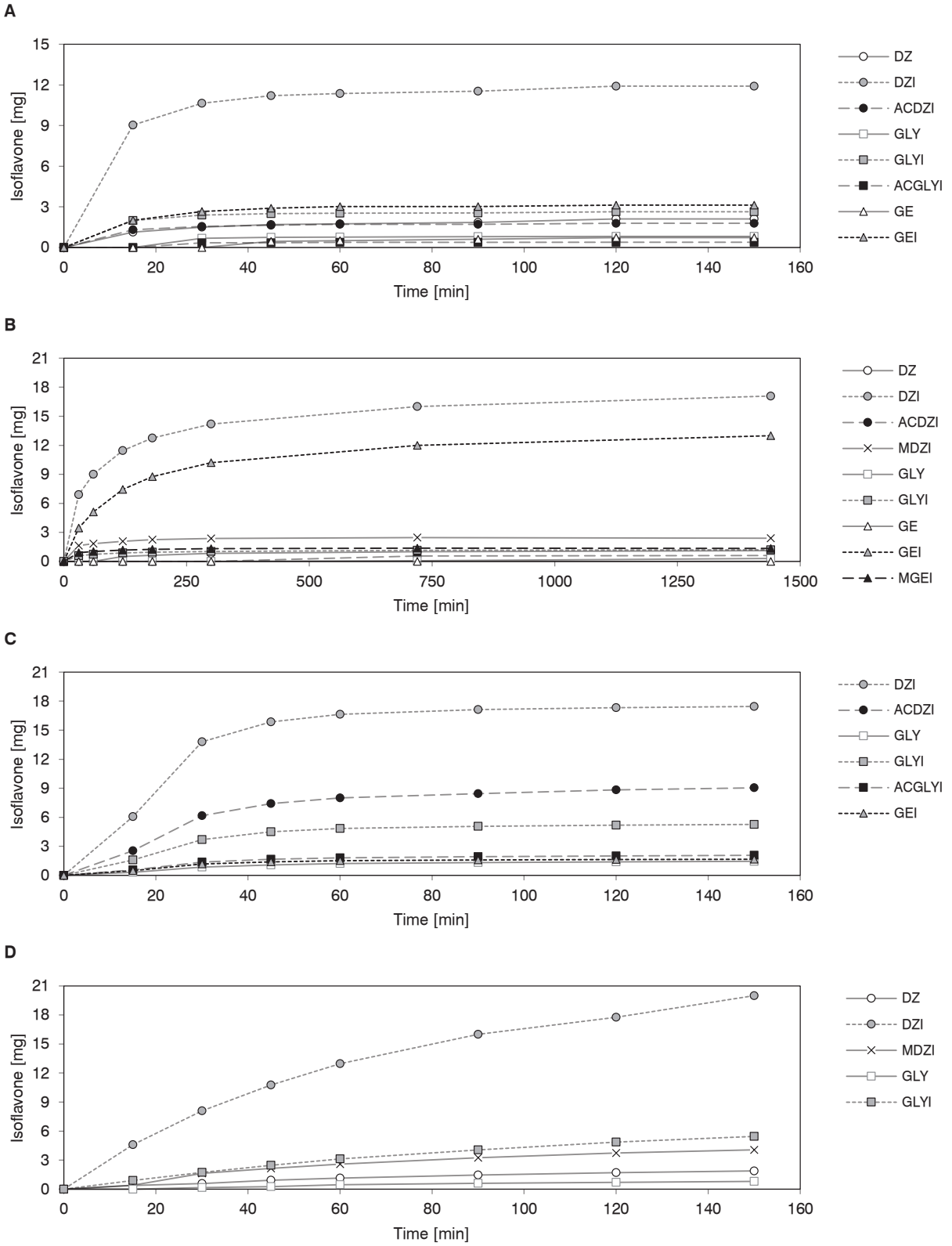

Fig. 3. Dissolution profiles of individual isoflavones per dosage unit in soya-based dietary supplements.

A – dissolution profile of sample S1, B – dissolution profile of sample S2, C – dissolution profile of sample S3, D – dissolution profile of sample S4.
Specification of samples is given in Tab. 1.
DZ – daidzein, DZI – daidzin, ACDZI – acetyl daidzin, MDZI – malonyl daidzin, GLY – glycitein, GLYI – glycitin, ACGLYI – acetyl glycitin, GE – genistein, GEI – genistin, MGEI – malonyl genistin.

Tab. 4. Contribution of individual isoflavones in solid formulation and final solution obtained by dissolution testing.

Contribution from total isoflavones	S1		S2		S3		S4	
	Final solution	Solid formulation	Final solution	Solid formulation	Final solution	Solid formulation	Final solution	Solid formulation
Daidzein [%]	32.7	9.0	2.5	3.0	NQ	NQ	6.2	5.9
Daidzin [%]	27.7	50.6	48.9	46.1	51.3	47.3	63.0	62.1
Acetyl daidzin [%]	3.4	7.6	1.4	1.7	18.9	24.5	NQ	NQ
Malonyl daidzin [%]	NQ	NQ	3.0	6.5	NQ	NQ	6.0	12.6
Glycitein [%]	2.4	3.5	0.7	0.8	4.8	3.9	1.9	2.5
Glycitin [%]	6.2	11.2	3.0	3.2	14.9	14.2	22.9	16.9
Acetyl glycitin [%]	0.9	1.7	NQ	NQ	4.6	5.6	NQ	NQ
Genistein [%]	18.6	3.1	0.6	NQ	NQ	NQ	NQ	NQ
Genistin [%]	8.0	13.3	38.1	35.1	5.5	4.5	NQ	NQ
Malonyl genistin [%]	NQ	NQ	1.8	3.6	NQ	NQ	NQ	NQ

NQ – not quantified.

portant in vitro predictor of oral bioavailability. The technique of microencapsulation of isoflavones with sodium-carboxymethylcellulose and gelatin was applied for enhancement of their in vitro dissolution properties [23]. Encapsulation of phenolic compounds also increased their stability and shelf life through protection from chemical damage. Additionally, incorporation to various nanocarriers (nanotubes, microemulsions, liposomes or micelles) is a method proposed for enhancement of bioavailability of isoflavones [27].

Furthermore, some other aspects concerning the production of dietary supplements should be taken into account. Selection of an appropriate extraction procedure is of great importance for isolation of active compounds from plant material [28]. Recent trends are mostly oriented towards development and application of green and sustainable extraction techniques for scientific and manufacturing purposes. Some of the newly proposed environmentally friendly methods for extraction of polyphenols include usage of deep eutectic solvents, microwaves, ultrasound, high hydrostatic pressure or enzymes [29–31]. Moreover, various extraction methods are able to selectively recover different groups of polyphenolic compounds [32]. The obtained extracts with target bioactive compounds from the plant material are further processed and used in production of the final dosage form. Additionally, these types of extracts have great potential for application in various food and cosmetic products. For instance, antioxidant-rich extracts have been used for preservation and improvement of sensory characteristics of food products as well as enhancement of cosmetic ingredients efficiency (e.g. as UV boosters, enhancing the UV irradiation absorption of chemical filters) [33, 34].

Overall, the presented recommendations for improvement of bioavailability and general quality of bioactive ingredients as well as their pharmaceutical formulations are of major importance for health-conscious consumers. Necessity for innovations in the food sector emerged especially during the pandemic and post-pandemic era bringing new technologies in bioactive components production, food safety and security [35].

CONCLUSIONS

The results obtained in this study showed that the quality of the analysed dietary supplements was highly variable and particularly impaired by inadequate isoflavone dissolution profile, content uniformity and discrepancies from the declared content of active compounds. The observed shortcomings may be explained by insufficient control during standardization of the plant extract, as well as by the poor control of the manufacturing process and of the final product. Bearing in mind the increased interest of consumers for supplements with health-promoting activity, particularly in the post-lockdown era, this issue is even more important [35]. Therefore, examination of chemical composition and dissolution properties of isoflavones-containing supplements should not be omitted. Additionally, to obtain high quality products, focus should be directed towards the development of adequate pharmaceutical formula-

tions. Since various approaches for improvement of biopharmaceutical properties of isoflavones have been developed, supported by scientific data, future trends should be directed towards implementation of these findings in production of commercial dietary supplements.

Acknowledgements

This work was a part of the project financed by the Ministry of Science and Technological Development, Republic of Serbia, No. TP31022 (contract number 451-03-68/2022-14/200114).

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Received 3 March 2023; 1st revised 21 March 2023; accepted 21 March 2023; published online 30 March 2023.