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# Soybean (*Glycine max* L.) isoflavones: Chemical composition and its chemometrics-assisted extraction and authentication

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**ABSTRACT**

Soybeans (*Glycine max* L.), a functional food widely consumed in Asia, has been reported as the main source of isoflavones. Phytoestrogen properties of soy isoflavones showed their activity as ligands for estrogen receptors and exhibited the estrogenic potency as reported in the previous *in vitro* and *in vivo* studies. Soy foods became most popular due to their benefits to human health and body function. The utilization of chemometrics in soybean isoflavones extraction and authentication was reported along with the increasing trends of computational analytical chemistry. A review on isoflavones contents in soybean, as well as its chemometrics-assisted extraction and authentication, is presented. This review aimed to report isoflavones contents in soybean, optimization designs for isoflavones extraction, and chemometrics algorithms for authentication purposes of soy-related products.

## INTRODUCTION

Isoflavonoids, a plant's secondary metabolites class, are flavonoid compounds produced by the Fabaceae family (Danciu *et al.*, 2017). Several plants were reported to be sources of isoflavones, such as *Pueraria lobata* (Kaufman *et al.*, 1997), *Medicago sativa* (Soto-Zarazúa *et al.*, 2016), *Vigna radiata* (Zaheer and Humayoun Akhtar, 2017), *Trifolium pratense* (Pakalapati *et al.*, 2009), and *Glycine max* (Liu *et al.*, 2014; Messina, 1999). Soybeans (*Glycine max* L.) are reported to be one of the main sources of dietary isoflavones and are well known as a healthy food with a high quality of protein, fatty acids, and other healthy components (Wang *et al.*, 2011). Soy products have been widely consumed in several Asian countries and are served as various kinds of food, such as tempeh, tofu, soymilk, miso, soy nuts, and many more (Messina *et al.*, 2006).

In two last decades, soy products were not only consumed as one of the functional foods but also were reported to provide several benefits on cardiovascular health, bone health, kidney function, cognitive function, mental health, skin health, reproduction function, thyroid function, and anticancer (Messina, 2016). The research interest on anticancer activities of soy isoflavones has increased in recent years due to their beneficial effects on the prostate cancer (Dong, 2011; Sivoňová *et al.*, 2018; Sugiyama *et al.*, 2013; Zhang *et al.*, 2016), breast cancer (Boucher *et al.*, 2013; Kang *et al.*, 2010; Yuliani *et al.*, 2016; Ziaei and Halaby, 2017), ovarian cancer (Lee *et al.*, 2012; Zhuang *et al.*, 2010), and colorectal cancer (Shafiee *et al.*, 2016; Yu *et al.*, 2016). Previous studies was reported that the biological activities of soy foods were linked to the presence of the soy isoflavones (Lissin and Cooke, 2000; Valsecchi *et al.*, 2011). The major soy isoflavones consist of isoflavone aglycones, namely, genistein, daidzein, and glycitein, and their glucosides (i.e., genistin, daidzin, and glycitin) (Křížová *et al.*, 2019; Yatsu *et al.*, 2016). It was also reported that the content percentage of genistein, daidzein, and glycitein werewas about 50%, 40%, and 10% from total isoflavone profiles, respectively (Hassan, 2013). However, the abundance of isoflavones in several soy products was varied due to their

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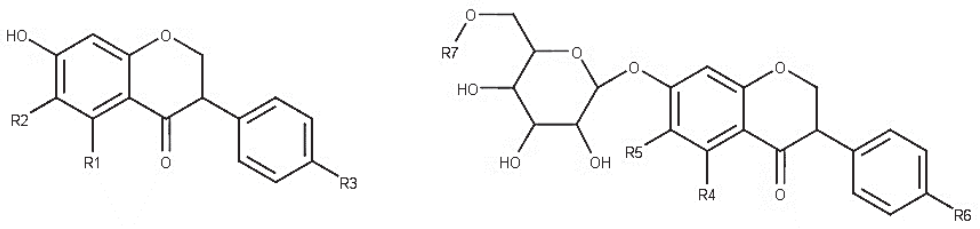
production or processing technique, as well as their extraction process (Anderson and Wolf, 1995; Erdman *et al.*, 2004; Yuliani *et al.*, 2019).

The conventional extraction method using one variable at a time approach can be developed in natural product extraction. Nevertheless, this approach was time-consuming and expensive for optimization (Banik and Pandey, 2008). It was important to develop an effective and more economical method for selecting extraction parameters, such as extraction duration, composition of solvent, pH, temperature, and solid to liquid (Anuar *et al.*, 2013; Borges *et al.*, 2011; Wijngaard and Brunton, 2010). Chemometrics, a combination method of mathematics and statistics to solve all types of chemical problems, can be applied in the method development (Miller and Miller, 2010). Chemometrics techniques were applicable in the optimization stage since the experimental design was stated as one of the specific areas in the chemometrics scope (Brereton, 2018). The advantage of applying experimental design in the optimization stage is the possibility to observe the interaction between different variables (Myers *et al.*, 2016). The design of the experiment can be carried out in natural product science, especially in the optimization process for extraction method with the application of response surface methodology (RSM) techniques, namely, full factorial design (FFD), Box–-Behnken design (BBD), central composite design (CCD), and Doehlert design (Riswanto *et al.*, 2019).

The employment of chemometrics in soy research was not only for the optimization process but also for the authentication process. Food authentication is described as the verification process to ensure that food or sample material complies with the label description, such as the origin, method, or processing technology used for the production (Danezis *et al.*, 2016). The trend of authentication research has been increasing since it was reported that the chemometrics of pattern recognition and multivariate analysis play an important role in plants identification, fingerprinting profiling, species discrimination, and metabolite analysis (Caligiani *et al.*, 2010; Gerbig *et al.*, 2017; Pompeu *et al.*, 2018; Wehrens, 2011). Principal component analysis (PCA) is a commonly used algorithm for dimensionality reduction since it provided a variance profile for each group in a set of multivariate data (Gromski *et al.*, 2015). Other chemometrics algorithms such as partial least square-discriminant analysis (PLS-DA) and cluster analysis (CA) were also applied in natural product analysis (Hong *et al.*, 2011; Shen *et al.*, 2018). Aiming to present integrative information of soybean isoflavones, this review discusses the chemical composition of isoflavones contained in soybeans, as well as chemometrics techniques for isoflavones extraction and authentication.

**Table 1.** Isoflavone structures and their glucosides.




(a) (b)

| Classification | Compounds | R1 | R2 | R3 | R4 | R5 | R6 | R7 |
|---|---|---|---|---|---|---|---|---|
| Aglycones (a) | Genistein | OH | H | OH | – | – | – | – |
| | Daidzein | H | H | OH | – | – | – | – |
| | Glycitein | H | $OCH_3$ | OH | – | – | – | – |
| | Formononetin | H | H | $OCH_3$ | – | – | – | – |
| | Biochanin A | OH | H | $OCH_3$ | – | – | – | – |
| Glucosides (b) | Genistin | – | – | – | OH | H | OH | H |
| | Daidzin | – | – | – | H | H | OH | H |
| | Glycitin | – | – | – | H | $OCH_3$ | OH | H |
| | Ononin | – | – | – | H | H | $OCH_3$ | H |
| | Sissotrin | – | – | – | OH | H | $OCH_3$ | H |
| | Acetylgenistin | – | – | – | OH | H | OH | $COCH_3$ |
| | Acetyldaidzin | – | – | – | H | H | OH | $COCH_3$ |
| | Acetylglycitin | – | – | – | H | $OCH_3$ | OH | $COCH_3$ |
| | Malonylgenistin | – | – | – | OH | H | OH | $COCH_2COOH$ |
| | Malonyldaidzin | – | – | – | H | H | OH | $COCH_2COOH$ |
| | Malonylglycitin | – | – | – | H | $OCH_3$ | OH | $COCH_2COOH$ |
| | Malonylononin | – | – | – | H | H | $OCH_3$ | $COCH_2COOH$ |
| | Malonylsissotrin | – | – | – | OH | H | $OCH_3$ | $COCH_2COOH$ |

Adapted from (Daems *et al.*, 2016; Křížová *et al.*, 2019).

## METHODS

This review article was accomplished by identifying, investigating, and assembling numerous review articles, original articles, and related books from reputable databases, such as Web of Science, PubMed, and Scopus. Supporting references from books, journal articles, and scientific reports were accessed utilizing facilities provided by Universitas Gadjah Mada, Indonesia. The literature investigation process was conducted between July and August 2020. The keywords explored during literature searching consisted of "*Glycine max* L.", "isoflavones", "soybean isoflavones content", "isoflavones anticancer activities", "experimental design of extraction", and "*Glycine max* L. authentication".

## CHEMICAL COMPOSITION

Isoflavones, a very important biologically active class of compounds contained in soybean, are recognized because of their benefits to human health (Ferreira *et al.*, 2011). Table 1 presents the structures of the isoflavones and their glucosides. Isoflavones can be categorized into aglycones and glucosides due to the presence of sugar moiety linked to hydroxyl groups (Yatsu *et al.*, 2016). In soybeans, there are 12 main isoflavones such as free aglycones (daidzein, genistein, and glycitein), their respective glucosides (daidzin, genistin, and glycitin), acetyl glucosides (acetyldaidzin, acetylgenistin, and acetylglycitin), and malonyl glucosides (malonyldaidzin, malonylgenistin, and malonylglycitin) (Rostagno *et al.*, 2007).

In previous studies, it was reported that the isoflavones content increased along with the occurrence of the fermentation process (Chaiyasut *et al.*, 2010; Rostagno *et al.*, 2007). The use of *Bacillus subtilis natto* (*B. natto*) successfully enhanced the concentration of genistein and daidzein eight times higher compared to those in the raw soybean (Hasim *et al.*, 2015). Other researchers reported that the content of genistein, daidzein, and glycitein increased within three3 days of fermentation with the utilization of *Rhizopus oligosporus* (Kuligowski *et al.*, 2017). Hence, fermentation products of soybeans, such as tempeh, attracted much attention and became popular to be studied in the last decade (Bavia *et al.*, 2012; Haron *et al.*, 2009, 2011; Jeleń *et al.*, 2013).

Soybeans isoflavones in several publications have been stated as flavonoids phytoestrogen (Hughes, 2003; Křížová *et al.*, 2019). Phytoestrogen was described as natural compounds from plants characterized by their molecular structure and size, resemblingd estrogen, especially estradiol, and showed estrogenic and/or antiestrogenic activities (Kurzer and Xu, 1997). Isoflavones have been linked to estradiol since these compounds showed the similarity of the structure to estradiol as a human hormone (Křížová *et al.*, 2019). Although isoflavones and estradiol structures showed several differences, the moiety of phenol groups enabled interaction with estrogen receptors (ER) and activated this receptor accordingly (Wang *et al.*, 2008). Figure 1 shows the similarity of structures and their phenolic/hydroxylic profiles of estradiol and three main isoflavone aglycones in soybeans.

According to previous publications on *in vitro* and *in vivo* studies, the estrogenic potency from the highest to the lowest was supposed as estradiol, genistein, glycitein, daidzein, formononetin, and biochanin A, respectively (Hughes, 2003). Soybean isoflavones, similar to other phytoestrogens, showed their

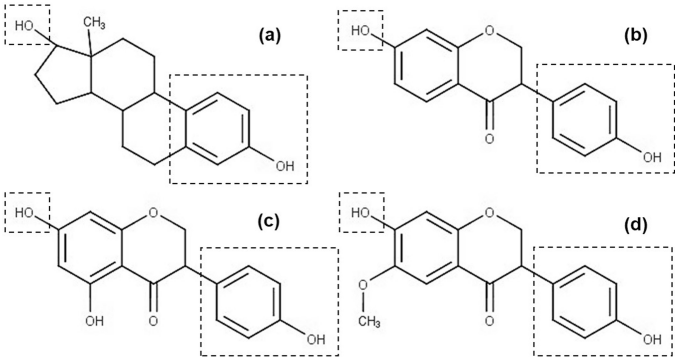



**Figure 1.** Structure similarities of estradiol (a) and three main isoflavone aglycones in soybeans, namely daidzein (b), genistein (c), and glycitein (d). The dotted lines indicate the phenolic and hydroxyl moieties.

activity as ligands for ER (Matsuda *et al.*, 2001; Messina *et al.*, 2006), a biomarker of breast cancer (Ali *et al.*, 2011; Shiau *et al.*, 1998). Among the soy isoflavones, genistein became the most popular studied compound since it plays an important role as a reference ligand for both estrogen receptor α (ERα) and estrogen receptor β (Helferich *et al.*, 2008; Istyastono *et al.*, 2017; Morito *et al.*, 2002). Genistein is also reported with its capability of crossing the blood–brain barrier and producing antioxidant activities against radiation of ultraviolet and chemicals (Zeng *et al.*, 2004).

## CHEMOMETRICS-ASSISTED ISOFLAVONES EXTRACTION

A modern approach to the optimization process was commonly conducted by employing a computational experimental design (Kleijnen, 2010). In natural product chemistry, experimental designs can be applied in analytical method development (Prabaningdyah *et al.*, 2017; Riswanto *et al.*, 2020; Siregar *et al.*, 2017), biological product enhancement (Farombi *et al.*, 2018; Managamuri *et al.*, 2017; Momen *et al.*, 2016; Srivastava *et al.*, 2018; Talluri *et al.*, 2019), and parameters selection for efficiency and economic consideration in biochemical processing (Ciechomska *et al.*, 2016; Ha *et al.*, 2018; Merib *et al.*, 2014; Xiong *et al.*, 2016).

The extraction process of soybean isoflavones can be carried out using several extraction techniques such as maceration, ultrasonication, filtration, agitation, and centrifugation. Extraction process optimization combined with experimental designs for isoflavones extraction can be conducted with RSM techniques, namely, FFD, simplex centroid design (SCD), CCD, and BBD. Table 2 presents the list of reported chemometrics designs and techniques in soybean isoflavone extraction studies.

FFD has been successfully applied for optimizing the process of extraction of genistein and daidzein from dried tempeh, a fermented product of soybean. Tempeh powder was macerated and extracted using a liquid–liquid extraction (LLE) technique. An optimal extraction condition was obtained with the usage of ethanol 96%, the particle size of 0.6 mm, and the extraction time of 270 minutes. Total genistein and daidzein achieved from the optimal method were 26.03 mg% and 19.42 mg%, respectively (Yuliani *et al.*, 2018). SCD and other types of RSM belong to a mixture experiment in which the variables are the mixture components and the responses are the formula of the proportions of

**Table 2.** The employment of chemometrics in soybean isoflavone extraction studies.

| Samples/Sources | Extraction Techniques | Designs | References |
|---|---|---|---|
| Tempeh | Maceration, LLE | FFD | (Yuliani *et al.*, 2018) |
| Soybeans (BRS 257 cultivar) | Ultrasonication, centrifugation, filtration | SCD | (Yoshiara *et al.*, 2012) |
| Soybean seeds (DT2010 cultivar) | Maceration | CCD | (Le *et al.*, 2019) |
| Soybeans (BRS 257 cultivar) | Agitation, centrifugation | CCD | (Yoshiara *et al.*, 2018) |
| Soybeans (JS 335 cultivar) | Leaching, membrane process, and LLE– | CCD | (Lakshmi *et al.*, 2013) |
| Soy flour | Agitation, fractionation | BBD | (Abdella *et al.*, 2018) |
| Black soybean variety VL Bhatt | CSE, MAE, EAE | BBD | (Kumar *et al.*, 2019) |
| Chinese soybean cheese | UAE | BBD | (Jianming *et al.*, 2013) |
| Soybean from the Guangdong Inspection and Quarantine Technology Center | QuEChERS method | BBD | (Ding *et al.*, 2016) |

LLE = liquid–liquid extraction; CSE = conventional solvent extraction; MAE = microwave-assisted extraction; EAE = enzyme-assisted extraction; UAE = ultrasound-assisted extraction; QuEChERS = quick, easy, cheap, effective, rugged, and safe; FFD = full factorial design; SCD = simplex centroid design; CCD = central composite design; BBD = Box–Behnken design.

each ingredient (Myers *et al.*, 2016). Yoshiara *et al.* (2012) carried out soy isoflavone extraction and optimized the solvent polarity by varying the composition of four solvents, namely, water, acetone, ethanol, and acetonitrile. After conducting extraction techniques, including ultrasonication, centrifugation, and filtration, the content of different soy isoflavones was stated as a response function and determined using a validated high-performance liquid chromatography (HPLC) method. It was found that malonyl-glycosidic and total forms can be optimally extracted using water : acetone : ethanol (2 : 1 : 1, by volume); glycosidic isoflavones were best extracted using water : acetone : acetonitrile (2 : 1 : 1, by volume), and the less polar aglycone forms were extracted with water : acetone (1 : 1, by volume) (Yoshiara *et al.*, 2012).

CCD, a type of RSM technique introduced by Box and Wilson (1951), was composed of three parts, including full factorial or fractional factorial design, star points, and center point (Box and Wilson, 1951). This design was successfully generated and applied for optimizing isoflavones extraction of soybean of DT2010 (Le *et al.*, 2019), BRS 257 (Yoshiara *et al.*, 2018) and JS 335 cultivar (Lakshmi *et al.*, 2013). Several optimization parameters, namely, solvent concentration, extraction time, reaction temperature, solvent-material ratio, and pH, can be defined as experimental factors to achieve optimized extraction conditions.

BBD became the most popular RSM technique for optimization since it was more efficient and economically effective than the other three-level experimental designs, mainly for a large number of variables (Bezerra *et al.*, 2008; Box and Behnken, 1960). This design has been widely used since there are no factorial or extreme points of experiments (Riswanto *et al.*, 2019). BBD can be applied to observe not only conventional extraction techniques such as agitation and fractionation (Abdella *et al.*, 2018) but also more sophisticated techniques for modern extraction studies. BBD has been applied in a comparison study of conventional solvent extraction (CSE), microwave-assisted extraction (MAE), and enzyme-assisted extraction (EAE) for extracting total phenolics and anthocyanins from the sample of black soybean variety VL Bhatt (Kumar *et al.*, 2019). Another modern extraction technique using ultrasound-assisted extraction (UAE) has been utilized by the BBD technique for determining total isoflavones content in cheese from Chinese soybean (Jianming *et al.*, 2013). The UAE technique proved to increase the extraction efficiency compared to the mix-stirring extraction for extracting isoflavone derivatives, such as daidzin, glycitin, genistein, and malonyl genistin from freeze-dried ground soybeans (Rostagno *et al.*, 2003). A quick, easy, cheap, effective, rugged, and safe (QuEChERS) method coupled with high-resolution liquid chromatography quadrupole times of flight mass spectrometry was developed to extract, identify, and quantify daidzein, glycitein, genistein, daidzin, glycitin, and genistin in soybeans from the Guangdong Inspection and Quarantine Technology Center. The extraction conditions of soybean isoflavones by using the QuEChERS method were achieved as follows: MgSO4 and NaCl of 1.0 and 0.25 g, respectively; acetonitrile and water ratio of 70 : 30 (*v/v*); and ultrasonic duration of 20 minutes (Ding *et al.*, 2016).

## CHEMOMETRICS-ASSISTED ISOFLAVONES AUTHENTICATION

In natural product and food analysis, the authentication process was more important and was addressed to ensure the quality and safety of the samples (Irnawati *et al.*, 2020, 2021; Putri *et al.*, 2021; Rohman *et al.*, 2011; Suhandy and Yulia, 2019). By combining statistical and mathematical techniques and chemistry data, chemometrics can be performed to observe data output from analytical instruments such as high-HPLC, liquid chromatography–mass spectrometry (LC-MS), infrared spectroscopy, and quadrupole time of flight mass spectrometer (QTOF-MS). Chemometrics algorithms including PCA, PLS-DA, CA, and partial least squares regression (PLSR) were employed for specific authentication purposes. Table 3 presents the listed reports of chemometrics algorithms and instrumentation used in soybean isoflavone authentication studies.

PCA algorithm has been widely used in almost all scientific disciplines and probably the most popular chemometrics technique in authentication analysis (Rohman and Putri, 2019). As a chemometrics technique, PCA was chosen to performcarry

**Table 3.** The employment of chemometrics in soybean isoflavone authentication studies.

| Samples/sources | Instruments | Chemometrics | Aim of the study | References |
|---|---|---|---|---|
| Soybean seeds BRS 25 cultivar | HPLC | PCA | Observing the relationship between the distributions of isoflavones to the various roasting treatments used to create the flour | Giaretta *et al*., 2015 |
| Soybeans | HPLC; QTOF-MS | PCA | Identifying the multivariate trends of isoflavone aglycones in soybeans treated by drying and storage process | Ferreira *et al*., 2019 |
| Soybeans | HPLC | PCA | Investigating the increase of isoflavones in the aglycone form | Miladinović *et al*., 2019 |
| 1168 soybean accessions | HPLC | PCA | Profiling the seed isoflavone composition of 1168 soybean accessions | Azam *et al*., 2020 |
| Wild soybeans, cultivated soybeans, and bean products | HPLC; LC-MS | PCA; CA | Developing an analytical method to evaluate the quality of wild soybean, cultivated soybean, and bean products | Hong *et al*., 2011 |
| Soybean mutants | Raman spectroscopic | PCA; CA | Demonstrating a reliable fast determination and discrimination between the mutants and control groups | Ogruc Ildiz *et al*., 2020 |
| 44 varieties of the soybean | HPLC | PLS-DA; CA | Metabolite profiling for assessing the quality of food soybeans from China, Japan, and Korea | Kim *et al*., 2014 |
| Chinese and Korean soybeans | FTIR | PLS-DA; PLSR; CA | Combining FTIR with chemometrics techniques for discriminating Chinese and Korean soybeans | Lee *et al*., 2018 |

PCA = principal component analysis; CA = cluster analysis; PLS-DA = partial least squares discriminant analysis; PLSR = partial least squares regression.

out the dimensionality reduction of the data when a correlation was present (Miller and Miller, 2010). The advantage of PCA is the possibility to generate principal components for further analysis or data visualization (Brereton, 2007). In soybean isoflavone analysis, PCA was used to observe the relationship between the distributions of isoflavones of soybean seeds BRS 25 cultivar (Giaretta *et al.*, 2015), identify the multivariate trends of isoflavone aglycones (Ferreira *et al.*, 2019), and investigate the increased content of isoflavone aglycones (Miladinović *et al.*, 2019) and isoflavones profiling (Azam *et al.*, 2020). HPLC was the commonly used analytical instrumentation since it was possible to result in a separation profile of the presence analytes in the matrix or mixture samples (Snyder *et al.*, 2010).

PCA can be coupled with other chemometrics algorithms, such as CA, a method for objects grouping into classes, so that similar objects are in the same class (Miller and Miller, 2010). This chemometrics technique combination resulted in good contribution to evaluate the quality of wild soybean, cultivated soybean, and bean products (Hong *et al.*, 2011). Another study by Ogruc Ildiz *et al.* (2020) was conducted by using Raman spectroscopy coupled with PCA and CA. The determination and discrimination were between the mutants and control group of soybeans (Ogruc Ildiz *et al.*, 2020).

PLS-DA was recognized as the most well-known classification procedure in chemometrics (Brereton and Lloyd, 2014). This chemometrics approach has been extensively employed in the field of metabolomics studies (Gomez *et al.*, 2018; Gromski *et al.*, 2015). Metabolite profiling using HPLC method combined with PLS-DA and CA for assessing the quality of food soybeans from China, Japan, and Korea has been successfully conducted (Kim *et al.*, 2014). The studyies by Lee *et al.* (2018) reported the employment of Fourier transform infrared (FTIR) spectroscopy in combination with PLS-DA and CA to discriminate between Chinese and Korean soybeans. In this study, the PLSR model was generated to determine the origin of soybeans using the appropriate wavenumber selection (Lee *et al.*, 2018).

It is interesting to explore more about the chemical compositions in soybeans and soybean products by carrying out research employed with chemometrics techniques. Our future research will be focused on the isoflavone aglycones analysis of soybean products. Chemometrics techniques will be applied to determination genistein, daidzein, and glycitein by generating predictive models for each compound compared to a validated chromatographic method, such as HPLC.

## CONCLUSION

Soybean is a valuable and powerful functional food due to its benefits to human health. Phytoestrogen activity of soybean foods was linked to estradiol since these isoflavone compounds showed a structural similarity. Isoflavones content in soybean also exhibit several biological activities on cardiovascular, bone, mental, and skin health, as well as improve cognitive, kidney, reproduction, and thyroid function.

Chemometrics of the experimental design can be applied in the soybean isoflavones extraction for selecting extraction conditions including solvent concentration, extraction time, reaction temperature, solvent-material ratio, and pH. RSM techniques of FFD, SCD, CCD, and BBD have been reported as a computational optimization approach in numerous papers. The authentication process of soybean isoflavones was carried out for assuring the quality and safety of soy foods. Analytical data collected from analytical instrumentations, such as HPLC, QTOF-MS, LC-MS, and infrared spectrophotometer, can be processed using appropriate chemometrics algorithms of PCA, PLS-DA, CA, and/or PLSR to provide useful information in the fields of analytical chemistry.

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## AUTHORS' CONTRIBUTIONS

Florentinus Dika Octa Riswanto carried out the literature search and drafted the manuscript. Abdul Rohman reviewed the initial draft of the manuscript and supervised the content in the field of chemometrics for both extraction and authentication. Suwidjiyo Pramono reviewed the initial draft of the manuscript and supervised a substantial part in the field of natural materials research and extraction techniques. Sudibyo Martono provided the conceptualization of the article and supervised the content in the field of pharmaceutical chemistry and instrumental analysis.

## CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

## FUNDING

None.

## ETHICAL APPROVAL

This study does not involve the use of animals or human subjects.

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