Food Chemistry 264 (2018) 135–141

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Micellar systems of aliphatic alcohol ethoxylates as a sustainable alternative to extract soybean isoflavones

Ezequiel R. Coscueta, Luciana Pellegrini Malpiedi, Bibiana Beatriz Nerli*

Instituto de Procesos Biotecnológicos y Químicos (IPROBYQ), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) - Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 570, (S2002LRK) Rosario, Argentina

ARTICLE INFO

Keywords:
Micellar systems
Isoflavone extraction
Genapol
Tergitol

ABSTRACT

Ethoxylated aliphatic surfactants belonging to the Genapol and Tergitol series were assessed as extraction systems of isoflavones. They showed good extraction properties when compared with different solvents, the Genapol X-080 exhibiting the best performance. Available commercial isoflavone pills were used, as a starting simple matrix, to determine the parameters that affect the extraction procedure. The temperature and the surfactant concentration showed to be factors that favored significantly the extraction performance. The application of optimized variables (Genapol X-080 11% m/m, pH 4.5; extraction temperature of 54 °C and extraction time of 60 min) on soybean flour (natural) allowed extracting 3.237 ± 0.173 mg of isoflavone per gram of treated flour. This result was three times what it was for methanol under identical conditions. Extraction with these micellar systems represents a sustainable alternative methodology for industrial purposes due to its low cost, biodegradability, non-toxicity and easy scaling up.

1. Introduction

For centuries, soybean has supplied most of the protein requirements in the Asiatic diet and since its industrialization, in the 1940s, it has also become a significant part of the Western human and animal diet (Fernandez-Lopez, Lamothe, Delample, Denayrolles, & Bennetau-Pelissero, 2016). Soybean is mainly used to produce oil and protein derivatives, however, it is increasingly considered as an important source of bioactive phytochemicals such as isoflavones, saponins, phenolic acids and protease inhibitors (Luthria, Biswas, & Natarajan, 2007). Isoflavones (IF) are present in soybean products under two chemical forms (Fig. S1, Supplementary Material): aglycones (i.e., daidzein, glycitein and genistein) and their β-glycosides (i.e., daidzin, glycitin and genistin). In addition, β-glycosides may be conjugated as malonyl/acetylglycosides (i.e., 6′-O-malonyldaidzin, 6′-O-acetyldaidzin, 6′-O-malonylglycitin, etc.) (Murphy et al., 1999). Among these phytochemicals, daidzein and genistein, have showed protective properties such as reducing the risk of cardiovascular disease, lowering rates of some types of cancer and preventing menopause symptoms (Dong, Xu, Sikes, & Wu, 2013; Filiberto et al., 2013; Park, Ju, Park, & Han, 2013). However, they have also been considered to be potent endocrine disruptors, due to their estrogen-mimetic activity, thus becoming potential triggers of reduced fertility (Fernandez-Lopez et al., 2016; Omoruyi, Kabiersch, & Pohjanvirta, 2013). In addition, high consumption of soy isoflavones in Asian-American children has been associated with an increased risk of Kawasaki disease (Portman, Navarro, Bruce, & Lampe, 2016). These controversial effects need of further investigations to be clarified. In the meantime, removing IFs from the general population diet and reserving them for specific applications is a proper conduct to be adopted (Bennetau-Pelissero, 2017). In this way, developing scalable processes for recovering IFs from soybean byproducts will allow both to obtain an IF-enriched extract, consumable by those people susceptive of its beneficial properties, and to render safer IF-free soybean derivatives for population sensitive to their adverse effects.

Solvent extraction has been widely used for IF recovering due to its high efficiency, simplicity and easy scaling up (Murphy, Barua, & Hauck, 2002; Xu & He, 2007). Aqueous methanol, ethanol, and acetone solutions are typical solvents used for this purpose (Rostagno, Palma, & Barroso, 2003). Recently, a wide range of new solvents non-toxic, non-inflammable and biodegradable are been evaluated in order to develop sustainable and green extraction methods (Bajkacz & Adamek, 2017). Among them, certain surfactants fulfill the mentioned properties thus representing an economical alternative to hazardous, expensive organic solvents. Surfactants are amphiphilic molecules with the ability to form aggregates, namely micelles, above a critical micelle concentration (CMC) (Yazdi, 2011). These assemblies can interact with either hydrophilic or lipophilic molecules through hydrophobic and dipolar

* Corresponding author.
E-mail address: bnerli@fbioyf.unr.edu.ar (B.B. Nerli).

https://doi.org/10.1016/j.foodchem.2018.05.015
Received 19 October 2017; Received in revised form 12 April 2018; Accepted 2 May 2018
Available online 08 May 2018

interactions and hydrogen bonding, these features being useful for separation purposes (Sharma, Kori, & Parmar, 2015). Several surfactants such as sodium dodecyl sulfate, Triton X-100, PEG 2000 and Brij 35 were evaluated to extract polyphenols from fruit juices (Hosseinzadeh, Khorsandi, & Hemmaty, 2013; Sharma et al., 2015). In a previous work (Cordisco, Haidar, Coscueta, Nerli, & Malpiedi, 2016), our research group demonstrated that aqueous micellar two-phase systems of Triton X-114 were capable of recovering the 93% of IFs from soybean flour with a purification factor of almost 10 under adequate conditions of time, concentration and temperature. Despite this good performance, the UV absorbance signals of Triton X-114, which overlap those of IFs, make the analytical evaluation of process progress difficult, this being a disadvantage at industrial scale. Other surfactants with similar extraction and eco-friendly properties but transparent at 240–280 nm spectral range would be desirable. Ethoxylated primary and secondary aliphatic alcohols such as those belonging to the Genapol and Tergitol series (Fig. S2, Supplementary Material) possess these characteristics. Particularly, aqueous micellar two-phase systems of Genapol X-080 (GX080) has been successfully applied to recover and quantify vitamins A and E from human serum (Sirimanne, Patterson, Ma, & Justice, 1998) and those formed by Tergitol 15-S-7 (Tg7) and Tergitol 15-S-9 (Tg9) have been used to extract polycyclic aromatic hydrocarbons from aqueous solutions (Alibrahim, 2014).

Regard to IF recovery, dissimilar results were reported. According to He et al. (2005) the micellar systems of GX080 showed to be successful at extracting daidzein from Puerariae radix, however, Luthria et al. (2007) found these systems to exhibit a poor performance at recovering total IFs from soybean under their experimental conditions. A complete study of these micellar systems, aided by rigorous statistical tools, would be required to understand these discrepant reports and to define whether the GX080 systems are adequate to extract isoflavones.

In this context, the goal of this work is to compare rigorously the efficiency of IF extraction by different solvents, focusing the attention in Genapol (GX080) and Tergitols (Tg7 and Tg9) as potential micellar extractants for industrial purposes. In addition, factors that affect significantly the extraction performance will be determined to optimize the process.

2. Experimental

2.1. Materials

The surfactants Genapol X-080 (GX080); Tergitol 15-S-7 (Tg7); Tergitol 15-S-9 (Tg9); Triton X-114 (TX114) and sodium dodecyl sulfate (SDS) were supplied from Sigma-Aldrich (St. Louis, MO, USA). Polyethylene glycols of molecular mass 8000 (PEG8000) and 600 (PEG600) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol (MetOH) and ethanol (EtOH) absolute were of HPLC grade. All the reagents were used as received without further purification. Isoflavone standards (Sigma-Aldrich, St. Louis, MO, USA) were dissolved in pure methanol until obtaining the following concentrations: daidzin 1.5–80.0 μg/mL; daidzein 0.3–17.0 μg/mL; genistin 0.7–40.0 μg/mL and genistein 0.2–13.0 μg/mL. All the other reagents were of analytical grade and used without further purification. Deionized water was used to prepare all the solutions.

Soy isoflavone tablets containing 79 mg IF/g were purchased at a local pharmacy.

Soybean flour was supplied by the food processing company Molinos Río de la Plata SA (San Lorenzo, Argentina).

2.2. Extraction procedures

The extraction efficiency of several solvents was determined by selecting commercial isoflavone tablets as solid matrix to minimize interferences. The pills were placed in a glass mortar and crushed with the pestle until obtaining homogenous fine particles. This powder was divided into fractions of 0.14 g which were introduced into the bottom of glass tubes. Then, 2 mL of the following solvents were added on each sample: water, ethanol (99 and 60% m/V), methanol, GX080 (1, 5 and 10% m/m), Tg7 (1, 5 and 10% m/m), Tg9 (1, 5 and 10% m/m), TX114 (5% m/m), SDS (5% m/m), PEG8000 (5% m/m), PEG600 (5% m/m) and NaOH 10 mM. The heterogeneous matrix-solvent systems were mixed vigorously for 30 min at a constant temperature (25.0 °C ± 0.1 °C) and then centrifuged in Eppendorf tubes at 12000 rpm for 10 min. The supernatants (extracts) were separated for analytical evaluation.

The performance of GX080 at recovering IFs from tablets (screening experiments and optimization, Section 2.4) was evaluated through the mentioned extraction procedure, carried out at several conditions of surfactant concentration (5–15% m/m), temperature (25.0–55.0 °C), pH (4.5–8.0) and incubation time (10–110 min).

Extraction of IFs from their natural matrix, the soybean flour, was also assessed. Different amounts of soybean flour were weighed and placed in glass tubes containing approximately 10 g of GX080 solution (10% m/m pH 4.5), thus leading to systems of flour/extractant ratios within the range 1–16% mass of soybean flour/mass of aqueous micellar system (F/AMS). After mixing vigorously for 60 min at a constant temperature (54.0 °C ± 0.1 °C), and centrifuging, the supernatants were separated for determination of total phenolic content and chromatographic analysis.

2.3. Analytical determinations

The total phenolic content (TPC) of extracts, was determined by the Folin–Ciocalteu colorimetric method (Singleton & Rossi, 1965) modified. The procedure consisted in mixing 30 μL of each supernatant (or its dilution if necessary) with 200 μL of the Folin-Ciocalteu reagent (diluted 1:10) and 100 μL of anhydrous sodium carbonate solution (7.4% m/V). After shaking thoroughly and incubating for 30 min at 25 °C, the absorbance of the resulting blue mixtures was measured at 765 nm. A calibration curve of gallic acid (0.025–0.200 mg/mL) was prepared in order to express the results as milligrams of gallic acid equivalents per milliliter of sample (mg GAE/mL). Incubation and absorbance measurements were performed on the Multiskan GO spectrophotometer (Thermo Fisher Scientific Corporation) operated using the SkanIt 3.2 software (Thermo Fisher Scientific Corporation).

Extracts with high TPC were also analyzed by thin layer chromatography (TLC). TLC was carried out by the one-way ascending technique using pre-coated plates with silica gel (Merck & Co.). The plates were developed in a solvent system of ethyl acetate/methanol/water (100:13.5:10), and then were dried and visualized under ultraviolet light (254 nm). The retention factor (Rf) of each spot was calculated and compared with those of standard daidzein, daidzin, genistein, and genistin. The densitometric chromatogram was obtained by acquiring a UV image of the plate with the UVP-Chromato-Vue C-75 cabinet and analyzing the spot intensity pattern with the aid of the software Fiji/ImageJ (Schindelin et al., 2012).

Optimized GX080 extract and methanol extract (as reference) were conveniently diluted (1:200) and analyzed by high-performance liquid chromatography (HPLC). The procedure was performed in a reverse phase column coupled with a guard column containing the same stationary phase (COSMOSIL 5C18-AR-II Packed Column – 4.6 mm I.D. × 250 mm). Separation of isoflavones was carried out by applying the operating conditions described previously (Cordisco et al., 2016). The separation analysis was performed using a Waters e2695 separation module system interfaced with a photodiode array UV/Vis detector (PDA 190–600 nm). The identification and quantification of daidzein, daidzin, genistein and genistin in the GX080 extract were performed by comparison of the retention times and the absorption spectra (peak area) with those corresponding to the standard of each isoflavone.

2.4. Statistical analysis

Each experiment was performed either in duplicate or triplicate and the results were expressed as the mean values with standard deviations (SD). The major statistical analysis was carried out with the aid of RStudio V 1.0.143.

2.4.1. Comparison of extraction efficiency

The mean TPC values were analyzed statistically by one-way analysis of variance (ANOVA) followed by the Tukey's post-hoc test (Kutner, Nachtsheim, Neter, & Li, 2005; Tukey, 1949). Separation of means was conducted by using the least significant difference at the 5% probability level.

2.4.2. Screening experiments

Those factors and interactions that affect significantly the extraction efficiency of GX080 were determined with the aid of a 2^4 full factorial design (4 = experimental factors). The parameters surfactant concentration (X_A); extraction time (X_B); pH (X_C) and temperature (X_D) were chosen as factors and the TPC in the supernatant was selected as the response (Y). Two codified levels (−1, +1) were assayed for each factor thus rendering a design consisted of 16 randomized experiments, two replicates of the complete design being carried out. The code and the real levels of factors are shown in Table 1. Response values (Y) from the experiments of the factorial design were fitted to the following polynomial linear model:

$$Y = \beta_0 + \beta_A X_A + \beta_B X_B + \beta_C X_C + \beta_D X_D + \beta_{A,B} X_A X_B + \beta_{A,C} X_A X_C + \beta_{A,D} X_A X_D + \beta_{B,C} X_B X_C + \beta_{B,D} X_B X_D + \beta_{C,D} X_C X_D + \varepsilon \quad (1)$$

where X_A, X_B, X_C and X_D are the coded levels of the independent variables above mentioned; β_0, β_i and β_{ij} are the regression coefficients for the intercept, linear and binary-interaction effects respectively; and ε, the residual error.

2.4.3. Optimization

The values of significant factors that maximized the extraction efficiency (TPC) of GX080 were determined by the response surface methodology (RSM) through a central composite design 2^2, consisted of four axial points and a total of 10 randomized experiments, three replicates of the complete design being carried out. The surfactant concentration (X_A) and the temperature (X_B) were selected as experimental factors while the pH and the extraction time were maintained constant at 4.5 (200 mM NaCit) and 60 min respectively. Table 2 shows the coded and the real levels of factors corresponding to this design. The response values (Y), i.e. the TPC in the supernatants from each treatment, were fitted to the following polynomial quadratic model:

$$Y = \beta_0 + \beta_A X_A + \beta_B X_B + \beta_{A,B} X_A X_B + \beta_{A,A} X_A^2 + \beta_{B,B} X_B^2 + \varepsilon \quad (2)$$

where X_A and X_B are the coded levels of GX080 concentration and temperature variables, β_0, β_i, β_{ij} and β_{ii} are the regression coefficients for the intercept, linear, binary-interaction and quadratic or curvature effects respectively; and ε, the residual error.

Table 1
Screening factorial design for 4 factors (2^4).

Exp.	Coded values				Natural values				TPC (mg GAE/mL)*
	X_A	X_B	X_C	X_D	X_A (% m/m)	X_B (°C)	X_C	X_D (min)	
1	−1	−1	−1	−1	5	25	4.5	10	0.58 ± 0.06
2	1	−1	1	−1	15	25	8	10	0.89 ± 0.14
3	1	−1	−1	−1	15	25	4.5	10	1.04 ± 0.05
4	−1	−1	1	−1	5	25	8	10	0.50 ± 0.05
5	1	1	−1	−1	15	55	4.5	10	1.23 ± 0.09
6	1	1	1	−1	15	55	8	10	1.01 ± 0.05
7	1	1	−1	1	15	55	4.5	110	1.11 ± 0.10
8	−1	−1	1	1	5	25	8	110	0.58 ± 0.06
9	1	1	1	1	15	55	8	110	1.19 ± 0.10
10	−1	1	1	1	5	55	8	110	0.70 ± 0.06
11	1	−1	1	1	15	25	8	110	0.98 ± 0.06
12	1	−1	−1	1	15	25	4.5	110	1.26 ± 0.08
13	−1	1	1	−1	5	55	8	10	0.69 ± 0.02
14	−1	1	−1	1	5	55	4.5	110	0.70 ± 0.07
15	−1	−1	−1	1	5	25	4.5	110	0.48 ± 0.08
16	−1	1	−1	−1	5	55	4.5	10	0.74 ± 0.05

Abbreviations: Concentration (X_A), temperature (X_B), pH (X_C) and time (X_D).
* Values expressed as mean ± SD for duplicates.

Table 2
Central composite factorial design for 2 factors. Abbreviations: concentration (X_A) and temperature (X_B).

Exp.	Coded values		Natural values		TPC (mg GAE/mL)*
	X_A	X_B	X_A (% m/m)	X_B (°C)	
1	−1, 414	0	3	40	0.61 ± 0.05
2	0	1, 414	10	54	1.26 ± 0.14
3	1, 414	0	17	40	1.11 ± 0.12
4	0	0	10	40	1.12 ± 0.10
5	1	−1	15	30	1.16 ± 0.12
6	0	0	10	40	1.11 ± 0.08
7	0	−1, 414	10	26	0.96 ± 0.06
8	−1	1	5	50	0.99 ± 0.04
9	−1	−1	5	30	0.68 ± 0.07
10	1	1	15	50	1.13 ± 0.12

* Values expressed as mean ± SD for triplicates.

3. Results and discussion

3.1. Comparison of IF extraction efficiency of different solvents

A set of different solvent systems was tested as IF extractants. It included traditional organic solvents such as methanol, ethanol (Luthria et al., 2007) and aqueous solutions of different ethoxylated surfactants. The TPC in the extracts, considered a measure of IF extraction efficiency, is represented in Fig. 1A. As expected, methanol and ethanol demonstrated to possess high extraction efficiency. However, systems formed by the aliphatic alcohol ethoxylates GX080, Tg7, and Tg9 (10% m/m) also showed to be effective extraction systems with TPC of 0.859 ± 0.051; 0.611 ± 0.057 and 0.611 ± 0.030 mg GAE/mL respectively, the GX080 evidencing the best performance. These values are several-fold higher than those obtained with pure water and aqueous NaOH solution. This behavior appears to be closely related to the amphiphilic character of surfactants and its ability to form micelles that can interact with either hydrophilic or lipophilic groups of IF molecule. It should be noticed that GX080, Tg7 and Tg9 "solutions" are actually "micellar heterogeneous systems", since the working concentrations (1–10% m/m) are far higher than their respective CMCs ($4.6\ 10^{-3}$; $3.9\ 10^{-3}$ and $4.5\ 10^{-3}$% m/m) according to the reports of Cordisco et al. (2016) and Tergitol manufacturers. When comparing with other surfactant systems (Fig. 1B), the extraction yield of mentioned aliphatic alcohol ethoxylates is about two-times that of TX114 and SDS and three times that of amphiphilic polymers PEG600 and PEG8000. TLC analysis of extracts allowed to detect at least four intense spots, whose Rfs confirmed the presence of daidzein, daidzin, genistein and genistin (Fig. S3, Supplementary Material).

By considering that the GX080 exhibited the best performance among the surfactants, it was selected for a further study.

3.2. Factors affecting IF extraction

According to literature, many variables affect the extraction process of a given substance from a solid matrix (Grumezescu & Holban, 2017),

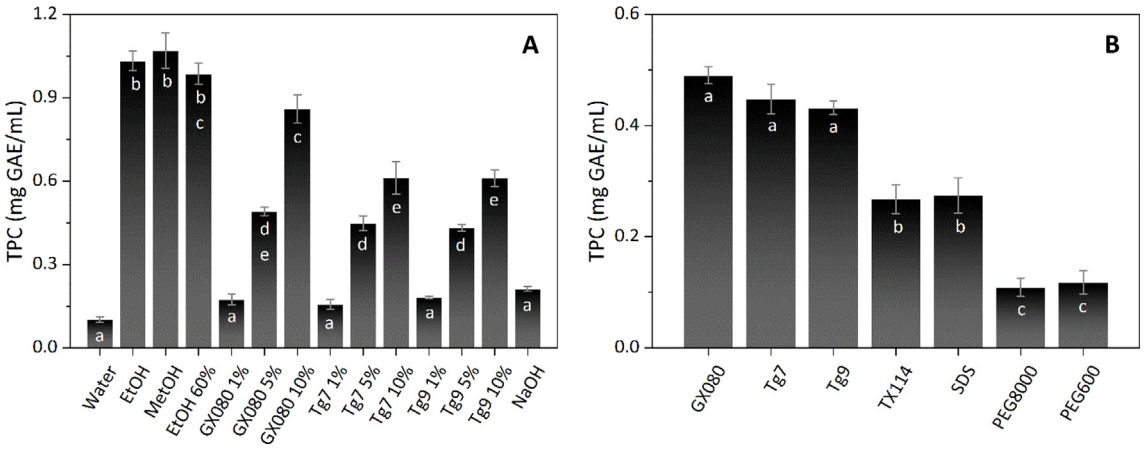

Fig. 1. Comparison of total polyphenolic content (TPC) in the extracts of: A) different solvents and aqueous micellar systems; B) different surfactant systems and amphiphilic polymer solutions. Bars represent means of two independent experiments. Error bars represent sample standard deviation (SD). The systems that share the same letter (a, b, c, d and e) do not present significant difference between their means ($P > 0.05$) according to the acquired data. Statistical analysis of the variance (One-Way ANOVA) was performed, with Tukey's post-hoc test.

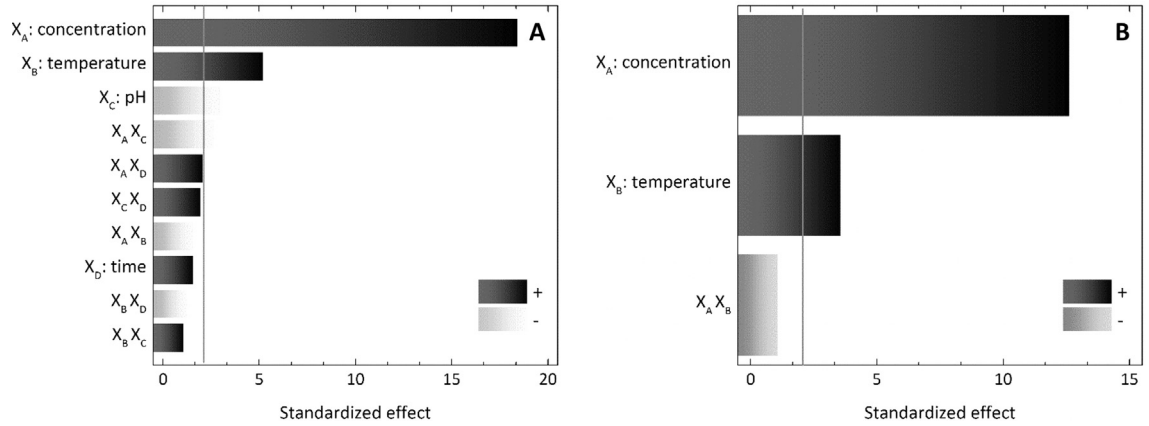

Fig. 2. Analysis of effects of the full factorial design at 2 levels. A) Pareto chart with effects of four experimental factors, in decreasing order of importance. B) Pareto chart with effects of the recalculated model for two factors, in decreasing order of importance. The grey lines represent the threshold of significance ($P = 0.05$).

i.e. temperature, extraction time, pH, the particle size of the solid, stirring rate and extractant concentration. A full factorial design was carried out to elucidate the most significant factors and interactions that determine the extraction performance in our case. Table 1 shows the design matrix and the results obtained for the 16 randomized experiments. The appropriate selection of maximum and minimum for the assessed factors was made according to our knowledge of the systems. The stability loss of flavonoids caused by high temperatures and long incubations, the drastic increase in medium viscosity with surfactant concentration were practical considerations that defined the experimental domain of each factor. An analysis of variance (ANOVA) was performed to determine which factors significantly affected the considered response (TPC) in the supernatants (Table S1, Supplementary Material). A graphical representation through a Pareto chart (Fig. 2) allowed the interpretation of data. Effects due to the chosen variables and their interactions on the response are represented by bars whose length is proportional to the effect magnitude. A line corresponding to the 5% of significance (P-value of 0.05) is placed as a reference.

Fig. 2A shows that GX080 concentration, temperature, and pH were the most significant effects on the TPC. Both temperature and GX080 concentration increased the TPC while pH caused the opposite behavior. The positive effect of GX080 can be understood by considering that at increasing surfactant concentrations, above the CMC, the micelle formation also increases thus enhancing the system capability of solubilizing flavonoids. Regard to the effect of temperature, as expected, it favored the extraction process. According to previous reports (Andrade, Mandarino, Kurozawa, & Ida, 2016; Grumezescu & Holban, 2017; Rickert, Meyer, Hu, & Murphy, 2004), a temperature raise decreases both surface tension and viscosity. The former helps the solvent wetting of the sample and the later increases diffusion and mass transfer of a given molecule from the solid matrix. In addition, increasing temperatures could contribute to disrupt interactions between the analyte and the matrix. In this work, the pH showed a slight negative effect on the response similarly to its interaction effect with GX080 concentration. This finding agrees with previous works (Cao et al., 2012; Cordisco et al., 2016) and may be caused by the polar character of IF molecule at more basic conditions which diminishes its solubility in micellar systems. The extraction time did not result to be a significant factor within the selected domain (10–110 min), this fact being advantageous since longer extractions could conduce to the degradation of certain compounds (Grumezescu & Holban, 2017). Thus, only three of the four factors studied proved to be relevant when modeling response behavior.

To describe the response dependence on the selected variables, the coefficients of the proposed linear model (Eq. (1) were estimated by the least square regression, thus resulting in the following expression:

$$Y = 0.85500 + 0.23375X_A + 0.06625X_B - 0.03750X_C + 0.02000X_D - 0.02000X_AX_B - 0.03375X_AX_C + 0.02625X_AX_D + 0.01375X_BX_C - 0.01625X_BX_D + 0.02500X_CX_D + \varepsilon \quad (3)$$

A significant relationship between the chosen variables and the

experimental response ($P < 0.05$) were obtained, as well as good R^2 value (0.9291) and adjusted R^2 (0.8954). However, a simpler model would be desirable for a further optimization step. Non-significant variables such as the extraction time effect and its interactions were neglected (terms in X_D of Eq. (1). In addition, pH and its interactions were also unconsidered (terms in X_C of Eq. (1) since their magnitudes were quite lower than those observed for temperature and GX080 concentration. Consequently, another linear model depending on GX080 concentration (X_A), temperature (X_B) and their interactions ($X_A X_B$) was proposed. The resulting equation with the recalculated coefficients is shown below:

$$Y = 0.85500 + 0.23375X_A + 0.06625X_B - 0.02000X_AX_B + \varepsilon \quad (4)$$

In this case, the R^2 and adjusted R^2 were 0.8609 and 0.8459 respectively. Although these values are lower than those observed previously they are still good enough to support the statistical validity and significance of the equation obtained. By weighing up pros and con of this recalculated model we considered that the loss in its representativeness was compensated by its higher simplicity. ANOVA results (Table S2, Supplementary material) indicated that both the surfactant concentration (X_A) and the temperature (X_B) had significant effects ($P < 0.05$) that favored the IF extraction while their interaction (X_AX_B) was not statistically significant. The representation of these results is shown in the Pareto chart of Fig. 2B.

3.3. Optimization of extraction procedure

The significant variables that affect the IF extraction, i.e. concentration and temperature, were optimized by applying the response surface methodology. A central composite design (CCD) was selected as it was described in Section 2.4. For the analysis, the pH was kept constant at 4.5 because higher pH values were observed to decrease the extraction efficiency. Besides, the soy proteins are sparingly soluble at this pH (Božič, Majerič, Denac, & Kokol, 2015), therefore, this condition would be appropriate to reduce the loss of protein if the extraction was applied on soybean flour.

The extraction time was fixed at an intermediate value of its domain interval, 60 min, since this factor did not show to affect significantly the response. According to the experimental data (Table 2), the following second order polynomial equation was obtained:

$$Y = 1.113580 + 0.165901X_A + 0.088039X_B - 0.084999X_AX_B - 0.125746X_A^2 + \varepsilon \quad (5)$$

This resulting model consists of two main positive effects, the surfactant concentration (X_A) and the temperature (X_B); one negative two-factor interaction effect (X_AX_B) and one negative curvature effect (X_A^2). The other curvature effect (X_B^2), proposed in Eq. (2), was excluded from this estimation since it was not significant ($P = 0.9615$). The R^2 and adjusted R^2 were 0.8592 and 0.8366 respectively thus expressing the good quality of the fit. ANOVA results are presented in Table 3 and the graphical representation, i.e. the response surface and contour of the calculated model, is shown in Fig. 3. As expected, the increase in both the surfactant concentration and the temperature led to higher TPC in the extracts. The decrease in the response, observed toward higher values of these factors, resulted from the negative X_AX_B and X_A^2 terms of Eq. (5). This behavior can be related to changes in the micellar structure (i.e. aggregation number and shape) that occur at higher surfactant concentration and temperature and affect the extraction capability (Yazdi, 2011).

According to this study, a maximal TPC of 1.24 mg GAE/mL in the extract is obtained by using GX080 11% m/m pH 4.5 at 54 °C for 60 min. These conditions are those of the experiment number 2 (pH 4.5; 54 °C, 60 min) of the central composite design (Table 2), except for the GX080 concentration which is 10% m/m instead of the optimized 11% m/m above mentioned. In this way, we selected this extract (from experiment 2) for a further analysis of IF content by HPLC. It should be noted that the GX080 extract did not require any treatment, previous to the HPLC, since this surfactant is compatible with the elution system. In addition, it does not absorb in the range 240–280 nm, thus facilitating the detection of IF by absorbance measurements. An extraction with methanol at the same conditions (at 54 °C for 60 min) was carried out in parallel for comparison. The total IF content determined in methanol and GX080 extracts (Table S3, Supplementary Material) was 83.3 ± 6.5 and 83.0 ± 12.3 mg per g of solid matrix (pill). These results lead to two important conclusions as follows: -the IF content in both extracts agrees with the IF content informed by the pill manufacturer (79 mg IF/g), thus indicating a complete extraction of IFs from pills and -the extraction performance of GX080 becomes comparable to that of methanol under optimal conditions.

Based on these promising findings, a further evaluation of GX080 micellar systems at recovering IF from the natural matrix, the soybean flour, was carried out.

Table 3
Analysis of variance (ANOVA) for the recalculated model of central composite experimental design.

Source	SS[a]	DF[b]	MS[c]	F	P
X_A	0.66045	1	0.660453	74.70	0.0000
X_B	0.18599	1	0.185993	21.04	0.0002
$X_A X_A$	0.26550	1	0.265504	30.03	0.0000
$X_A X_B$	0.08670	1	0.086699	9.81	0.0050
Lack of fit	0.01084	4	0.002709	0.31	0.8704
Pure error	0.18566	21	0.008841		
Total SS	1.39514	29			

$P < 0.05$ was considered significant.
[a] SS, sum of squares.
[b] DF, degrees of freedom.
[c] MS, mean squares.

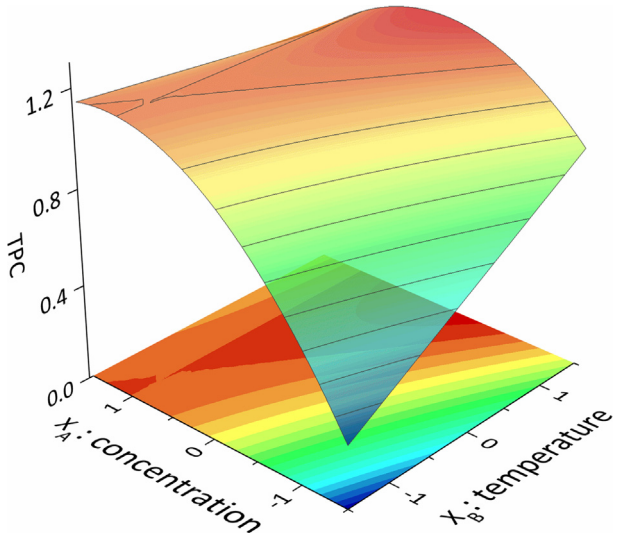

Fig. 3. Graphical representation of the adjusted model obtained with a composite central experimental design. Response surface and contour of the calculated model, expressing TPC as a function of concentration (X_A) and temperature (X_B), in coded values.

3.4. Recovering IF from soybean flour with GX080 systems

Solid to solvent ratio is known to be a parameter that affects the extraction process (Grumezescu & Holban, 2017). An adequate mass proportion of matrix-solvent (extraction system) is required to solubilize the target molecule and transfer it from the solid to the exterior. When analyzed this factor, a decreased TPC in the extracts was

observed at higher percentages of flour/GX080 system. Systems with 1 and 2% m/m of flour led to the highest total phenol recoveries. The last mass ratio (2%) was selected for further analysis since it allowed duplicating the treated solid mass for a given amount of solvent without a significant loss in the extraction efficiency (Fig. S4, Supplementary Material).

The extraction performance of methanol and GX080 (11% m/m, pH 4.5) system on soybean flour (2% m/m) were assessed at the optimal conditions (54 °C, 60 min). Our results showed that the extraction efficiency of the micellar system was far higher than that of methanol. The TPC in GX080 extract (4.854 ± 0.822 mg GAE/g flour) was approximately four-fold that corresponding to the methanol (1.342 ± 0.264 mg GAE/g flour). The enhanced extraction efficiency of GX080 systems in comparison to methanol could be attributed to the unique properties of micellar systems which facilitate all the steps involved in the mechanism of solid-liquid extraction. The overall process comprises -the contact solvent-solid matrix, -the disruption of solute-solid matrix interactions and -the formation of solute-solvent interactions which result in the solute solubilization and its removal from the solid matrix. Surfactants are known to reduce the surface tension of water, thus favoring the wetting of solid matrix and the accessibility of solvent into the particles of soybean flour. Methanol and other alcohols (glycerol, ethanol) have the opposite effect, i.e. they increase the surface tension, thus decreasing the contact between the solvent and the solid matrix. Additional differences are expectable for the behavior of GX080 and methanol at weakening/formatting interactions IF-solid matrix/IF-solvent. According to previous works IFs are present in soybean flour associated to the proteins glycinin and β-conglycinin (Jankowiak, Kantzas, Boom, & Van Der Goot, 2014). Methanol was reported to possess the ability to denature these proteins and to form aggregates which interact strongly with isoflavones and reduce their availability (Mahesha, Singh, Srinivasan, & Appu Rao, 2006). Besides, it has a tendency to form complex with the polar polyphenols, thus leaving non-polar or amphiphilic components in the matrix (Sharma et al., 2015). Conversely, micellar systems are able to establish both hydrophilic and hydrophobic forces with polyphenols due to their amphiphilic properties (Hosseinzadeh et al., 2013). During the surfactant self-assembling, the hydrocarbon/water contacts tend to minimize but some methylene groups (of GX080) remain exposed to solvent defining a palisade layer at the edge of the hydrophobic core of micelle. Probably, non-polar region of IF molecule interacts with this layer while their hydroxyl groups form dipole-dipole or hydrogen bonding interactions with the ethylene oxide chains belonging to the hydrophilic micelle surface (Taechangam, Scamehorn, Osuwan, & Rirksomboon, 2009). We speculate that this double interaction (hydrophobic/hydrophilic) is strong enough to drive the solubilization of IF in the surfactant system and its dissociation from the soybean proteins.

The IF analysis of each extract by HPLC (Table S4, Supplementary Material) demonstrated that the IF yield for the GX080 system (3.237 ± 0.173 mg of IF/g of flour) was higher than that of methanol (1.138 ± 0.151 mg of IF/g of flour). In both cases, the content of aglycones was lower than that of β-glycoside forms, this feature being in correspondence with the IF composition reported for other soy foods (Baú & Ida, 2015). In addition, the GX080 system exhibited certain selectivity to recover daidzein since its extract contained six-fold the amount of daidzein present in methanol extract. This is a remarkable fact when considering the enhanced bioactivity of aglycones respect to other conjugated forms. It is pertinent to mention that this excellent performance was observed by the mere application of conditions optimized for a simple matrix (i.e. IF pill powder) to a more complex one such as the soybean flour. A further optimization step, carried out with the actual matrix should be done, the expectable results being even better.

4. Conclusions

In this study, micellar systems formed by ethoxylated surfactants such as the GX080 demonstrated to possess a significant ability to extract isoflavones. Commercial IF pills were used as a starting simple matrix in order to determine variables that affect the extraction procedure by means of rigorous statistical tools. Optimized conditions determined in this step (GX080 11% m/m pH 4.5, extraction at 54 °C for 60 min) were then applied to the actual matrix, i.e. soybean flour, thus leading to an extraction efficiency of the micellar system far higher than that of methanol, a traditional solvent. The ability of surfactants of penetrating into the pores of the matrix, affecting the solute-matrix interaction and increasing the fluid-solid contact area would be the main causes of their extraction efficacy, the micellar systems becoming potentially usable in a wide range of solid-liquid extractions. Besides, the possibility of replacing organic solvents with biodegradable, non-inflammable, non-toxic surfactants and the potential application in large-scale extraction procedures make these systems suitable for industrial purposes. This assertion would not be restricted to the GX080 surfactant, since other similar systems, such as those formed by Tergitol surfactants, could achieve a comparable extraction performance after a proper optimization procedure.

Acknowledgements

This work was supported by a grant from Consejo Nacional de Investigaciones Científicasy Técnicas (CONICET) Argentina PIP0505/15. Author E.R. Coscueta acknowledges CONICET for PhD fellowship.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.foodchem.2018.05.015.

References

Alibrahim, M. (2014). Cloud point extraction of polycyclic aromatic hydrocarbons in aqueous solution with nonionic surfactants. *Tenside, Surfactants, Detergents, 51*(4), 333–338. http://dx.doi.org/10.3139/113.110315.

Andrade, J. C., Mandarino, J. M. G., Kurozawa, L. E., & Ida, E. I. (2016). The effect of thermal treatment of whole soybean flour on the conversion of isoflavones and inactivation of trypsin inhibitors. *Food Chemistry, 194*, 1095–1101. http://dx.doi.org/10.1016/j.foodchem.2015.08.115.

Bajkacz, S., & Adamek, J. (2017). Evaluation of new natural deep eutectic solvents for the extraction of isoflavones from soy products. *Talanta, 168*, 329–335. http://dx.doi.org/10.1016/j.talanta.2017.02.065.

Baú, T. R., & Ida, E. I. (2015). Soymilk processing with higher isoflavone aglycone content. *Food Chemistry, 183*, 161–168. http://dx.doi.org/10.1016/j.foodchem.2015.03.026.

Bennetau-Pelissero, C. (2017). Response to the letter from Dr Messina and Dr Badger following the publication of the paper by Fernandez-Lopez A, Lamothe V, Delample M, Denayrolles M and Bennetau-Pelissero C. *Food Chemistry, 225*, 293–301. http://dx.doi.org/10.1016/j.foodchem.2017.01.033.

Božič, M., Majerič, M., Denac, M., & Kokol, V. (2015). Mechanical and barrier properties of soy protein isolate films plasticized with a mixture of glycerol and dendritic polyglycerol. *Journal of Applied Polymer Science, 132*(17), http://dx.doi.org/10.1002/app.41837.

Cao, Y., Xing, H., Yang, Q., Bao, Z., Su, B., Yang, Y., & Ren, Q. (2012). Separation of soybean isoflavone aglycone homologues by ionic liquid-based extraction. *Journal of Agricultural and Food Chemistry, 60*(13), 3432–3440. http://dx.doi.org/10.1021/jf3003009.

Cordisco, E., Haidar, C. N., Coscueta, E. R., Nerli, B. B., & Malpiedi, L. P. (2016). Integrated extraction and purification of soy isoflavones by using aqueous micellar systems. *Food Chemistry, 213*, 514–520. http://dx.doi.org/10.1016/j.foodchem.2016.07.001.

Dong, X., Xu, W., Sikes, R. A., & Wu, C. (2013). Combination of low dose of genistein and daidzein has synergistic preventive effects on isogenic human prostate cancer cells when compared with individual soy isoflavone. *Food Chemistry, 141*(3), 1923–1933. http://dx.doi.org/10.1016/j.foodchem.2013.04.109.

Fernandez-Lopez, A., Lamothe, V., Delample, M., Denayrolles, M., & Bennetau-Pelissero, C. (2016). Removing isoflavones from modern soyfood: Why and how? *Food Chemistry, 210*, 286–294. http://dx.doi.org/10.1016/j.foodchem.2016.04.126.

Filiberto, A. C., Mumford, S. L., Pollack, A. Z., Zhang, C., Yeung, E. H., Perkins, N. J., ... Schisterman, E. F. (2013). Habitual dietary isoflavone intake is associated with decreased C-reactive protein concentrations among healthy, 900–906. https://doi.org/

10.3945/jn.112.173187.among.
Grumezescu, A. M., & Holban, A. M. (2017). *Ingredients extraction by physicochemical methods in food.* Elsevier Science.
He, J., Zhao, Z., Shi, Z., Zhao, M., Li, Y., & Chang, W. (2005). Analysis of isoflavone daidzein in puerariae radix with micelle-mediated extraction and preconcentration. *Journal of Agricultural and Food Chemistry, 53*(3), 518–523. http://dx.doi.org/10.1021/jf048545q.
Hosseinzadeh, R., Khorsandi, K., & Hemmaty, S. (2013). Study of the effect of surfactants on extraction and determination of polyphenolic compounds and antioxidant capacity of fruits extracts. *PLoS One, 8*(3), 1–7. http://dx.doi.org/10.1371/journal.pone.0057353.
Jankowiak, L., Kantzas, N., Boom, R., & Van Der Goot, A. J. (2014). Isoflavone extraction from okara using water as extractant. *Food Chemistry, 160*, 371–378. http://dx.doi.org/10.1016/j.foodchem.2014.03.082.
Kutner, M. H., Nachtsheim, C. J., Neter, J., & Li, W. (2005). *Applied linear statistical models* (5th ed). McGraw Hill.
Luthria, D. L., Biswas, R., & Natarajan, S. (2007). Comparison of extraction solvents and techniques used for the assay of isoflavones from soybean. *Food Chemistry, 105*(1), 325–333. http://dx.doi.org/10.1016/j.foodchem.2006.11.047.
Mahesha, H. G., Singh, S. A., Srinivasan, N., & Appu Rao, A. G. (2006). A spectroscopic study of the interaction of isoflavones with human serum albumin. *FEBS Journal, 273*(3), 451–467. http://dx.doi.org/10.1111/j.1742-4658.2005.05071.x.
Murphy, P. A., Barua, K., & Hauck, C. C. (2002). Solvent extraction selection in the determination of isoflavones in soy foods. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences, 777*(1–2), 129–138. http://dx.doi.org/10.1016/S1570-0232(02)00342-2.
Murphy, P. A., Song, T., Buseman, G., Barua, K., Beecher, G. R., Trainer, D., & Holden, J. (1999). Isoflavones in retail and institutional soy foods. *Journal of Agricultural and Food Chemistry, 47*(7), 2697–2704. http://dx.doi.org/10.1021/jf981144o.
Omoruyi, I. M., Kabiersch, G., & Pohjanvirta, R. (2013). Commercial processed food may have endocrine-disrupting potential: Soy-based ingredients making the difference. *Food Additives and Contaminants: Part A, 30*(10), 1722–1727. http://dx.doi.org/10.1080/19440049.2013.817025.
Park, M.-H., Ju, J.-W., Park, M., & Han, J. (2013). Daidzein inhibits carbohydrate digestive enzymes in vitro and alleviates postprandial hyperglycemia in diabetic mice. *European Journal of Pharmacology, 712*(1), 48–52. http://dx.doi.org/10.1016/j.ejphar.2013.04.047.
Portman, M. A., Navarro, S. L., Bruce, M. E., & Lampe, J. W. (2016). ScienceDirect Soy isoflavone intake is associated with risk of Kawasaki disease. *Nutrition Research, 36*(8), 827–834. http://dx.doi.org/10.1016/j.nutres.2016.04.002.
Rickert, D. A., Meyer, M. A., Hu, J., & Murphy, P. A. (2004). Effect of extraction pH and temperature on isoflavone and saponin partitioning and profile during soy protein isolate production. *Journal of Food Science, 69*(8), C623–C631. http://dx.doi.org/10.1111/j.1365-2621.2004.tb09910.x.
Rostagno, M. A., Palma, M., & Barroso, C. G. (2003). Ultrasound-assisted extraction of soy isoflavones. *Journal of Chromatography A, 1012*(2), 119–128. http://dx.doi.org/10.1016/S0021-9673(03)01184-1.
Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., ... Cardona, A. (2012). Fiji: An open-source platform for biological-image analysis. *Nature Methods, 9*(7), 676–682. http://dx.doi.org/10.1038/nmeth.2019.
Sharma, S., Kori, S., & Parmar, A. (2015). Surfactant mediated extraction of total phenolic contents (TPC) and antioxidants from fruits juices. *Food Chemistry, 185*, 284–288. http://dx.doi.org/10.1016/j.foodchem.2015.03.106.
Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*(3), 144–158.
Sirimanne, S. R., Patterson, D. G., Ma, L., & Justice, J. B. (1998). Application of cloud-point extraction–reversed-phase high-performance liquid chromatography. *Journal of Chromatography B: Biomedical Sciences and Applications, 716*(1), 129–137. http://dx.doi.org/10.1016/S0378-4347(98)00287-4.
Taechangam, P., Scamehorn, J. F., Osuwan, S., & Rirksomboon, T. (2009). Effect of nonionic surfactant molecular structure on cloud point extraction of phenol from wastewater. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 347*(1–3), 200–209. http://dx.doi.org/10.1016/j.colsurfa.2009.04.005.
Tukey, J. W. (1949). Comparing individual means in the analysis of variance. *Biometrics, 5*(2), 99–114. http://dx.doi.org/10.2307/3001913.
Xu, H. N., & He, C. H. (2007). Separation and purification of puerarin with solvent extraction. *Separation and Purification Technology, 56*(3), 397–400. http://dx.doi.org/10.1016/j.seppur.2007.06.003.
Yazdi, A. S. (2011). Surfactant-based extraction methods. *TrAC – Trends in Analytical Chemistry, 30*(6), 918–929. http://dx.doi.org/10.1016/j.trac.2011.02.010.