Pressurized water extraction of isoflavones by experimental design from soybean flour and Soybean Protein Isolate

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ABSTRACT

A Doehlert experimental design was conducted and surface response methodology was used to deter mine the effect of temperature, contact time and solid liquid ratio on isoflavone extraction from soybean flour or Soybean Protein Isolate in pressurized water system. The optimal conditions conducted gave an extraction yield of 85% from soybean flour. For Soybean Protein Isolate compared to soybean flour, the isoflavone extraction yield is 61%. This difference could be explained by higher aglycon content, while aglycon appears to be the least extracted isoflavone by pressurized water. The solid liquid ratio in the ASE cell was the overriding factor in obtaining high yields with both soybean products, while tempera ture has less influence. A high temperature causes conversion of the malonyls glucosides and glucosides isoflavone derivatives into glucosides or aglycons forms. pressurized water extraction showed a high sol ubilization of protein material up to 95% of inserted Soybean Protein Isolate.

1. Introduction

Soybean has been one of the most important sources of vegetable sourced protein in Asian civilizations for thousands of years, with many traditional products like tempeh, tofu or miso. Different soybean protein products from soybean flour (SF) to iso lated proteins have been developed by specific processing over the last decades. Soybean Protein Concentrates (SPC) with over 70% protein and Soybean Protein Isolates (SPIs) with over 90% protein are widely integrated in food products or directly consumed in soy milk or dietary products (Barnes, 2010). Isoflavones are the main secondary metabolites present in soybean and derivative products. Their structure is a combination of three basic molecular forms including genistein, daidzein and glycitein with four deriva tives per forms giving a totality of 12 isoflavones. Isoflavones are known to promote estrogenic activity in humans due to their similarity with the hormone β estradiol, (Wuttke, Jarry, & Seidlová Wuttke, 2007). A plethora of articles have been published on the effects of isoflavones on health. Some authors have demon strated the positive effects of isoflavones on the improvement of bone mineral density (Marini et al., 2008), the reduction of meno pausal symptoms, (Jou et al., 2008) and the inhibition or retarding of prostate cancer (Yan & Spitznagel, 2009). Consequently, isoflavone extraction is of interest in the production of dietary supplements from soybean products.

Isoflavone extraction is generally carried out for analytical pur poses with aqueous acetonitrile (Eldridge, 1982; Song, Barua, Buseman, & Murphy, 1998) which could also be mixed with DMSO, (Collison, 2008) or extracted with aqueous alcohol (Klump, Allred, MacDonald, & Ballam, 2001). Mixing techniques such as stirred extraction, shaking, vortexing, sonication, stirring, soxhlet and pressurized extraction have been compared in the literature (Luthria, Biswas, & Natarajan, 2007). Nevertheless, no industrial methodology has been reported because these solvents have not been approved for the food industry.

Pressurized water extraction (PWE) is the subject of increasing interest in the extraction of natural compounds (Mustafa & Turner, 2011). This is mainly due to subcritical conditions reached in the pressurized systems, which change the water properties conferring the ability to extract a wide range of compounds with or without less organic solvents (Carr, Mammucari, & Foster, 2011). Very few studies have showed the efficiency of PWE in the extraction of isoflavones from soybean. Despite this, to date only two studies have been reported with Accelerate Solvent Extraction system, which demonstrates the efficiency of Pressurized Liquid Extraction in the extraction of soybean isoflavones using either 70% EtOH in water (Rostagno, Palma, & Barroso, 2004), or with the addition of 5% DMSO (Luthria et al., 2007). The extraction by water tested in these works only gave a total isoflavone extraction yield of

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approximately 23.6% and 18.2% respectively. These studies identi fied the effects of Pressurized Liquid Extraction parameters like temperature, contact time and solid liquid ratio (introduced quan tity into the extractor cell with a fixed volume delivered) on the extraction of the various isoflavone derivatives, but extraction with water was not optimized. A low proportion of introduced amount was recommended because the liquid solid ratio had a strong impact. Degradation of glucoside and malonyl forms was observed with the temperature increase. This change in the soybean isofla vone derivative profile was also demonstrated in a High Pressure Processing system using water (Jung, Murphy, & Sala, 2008). A lack of optimization approaches in the literature is highlighted for the total isoflavone extraction yields concerning PWE. One study focused on the PWE optimization of soybean isoflavones in a 2L autoclave, compared to Soxhlet extraction with 80% MeOH. This method extracted 93% of the isoflavones (Li Hsun, Ya Chuan, & Chieh Ming, 2004) but malonyl derivatives were not analysed and these compounds could represent the majority of soybean iso flavones. Moreover, the role of protein content during this extrac tion method is crucial because PWE causes significant protein degradation by enhanced hydrolysis, which has been described in other vegetable protein. In rice protein this hydrolysis occurs in three steps: aggregation of proteins, degradation of proteins to polypeptides and finally hydrolysis to amino acids. Consequently, the protein matrix is greatly modified during PWE (Pourali, Asghari, & Yoshida, 2009, 2010; Sunphorka, Chavasiri, Oshima, & Ngamprasertsith, 2012).

The objective of this study was to optimize isoflavone extrac tion with pressurized water by the study of the influence of the various operating conditions (time, liquid/solid ratio) and the potentiality of soybean isoflavones PWE, while considering the influence of the operating conditions of the extracted material, with a mid range protein content for soybean flour (SR) and high protein content for Soybean Protein Isolate (SPI), containing approximately the same level of isoflavones. The trials were con ducted through a Doehlert experimental design on both materials in order to define the optimal conditions according to a response surface methodology. Furthermore, the goal of this study was to describe the changes that occur during Pressurized Extraction on the isoflavone profile.

2. Materials and methods

2.1. Reagents and materials

HPLC grade solvent acetonitrile, methanol, formic and chloridric acids were purchased from SIGMA ALDRICH. For the extractions, demineralized water was used and MilliQ water was used for the HPLC analysis. Isoflavone standards were purchased from EXTRA SYNTHESE. The soybean flour was purchased from LUP'INGREDI ENTS composed of 42% proteins, 3% ash and 8.5% moisture, the Soybean Protein Isolate used for the extraction study was SUPRO® XT 219D IP, purchased from SOLAE™ composed of 83% proteins, 6.2% ash and 6.3% moisture.

2.2. Conventional extraction

Analysis was performed on the initial solid material to measure initial isoflavone amounts from the material. An extraction analy sis based on acidic aqueous acetonitrile solution from Murphy's previous work (Song et al., 1998) was conducted in an Erlenmeyer flask with magnetic stirring as followed: 2 g of dried sample with 10 ml of acetonitrile, 2 ml of chloridric acid 0.1 M and 7 ml of dem ineralized water stirred for 2 h in a 100 ml flask. After a centrifuga tion at 6000*g* the supernatant was filtered through a PTFE membrane 0.22 μm.

2.3. ASE extraction

A Dionex™ ASE™ 350 system, which can reach subcritical water conditions with a fixed pressure of 1500 psi was used for this study. All extractions were conducted in a 10 ml stainless steel extraction cell. The material was mixed with 10 g of sand as an inert dispersing agent to avoid protein aggregation. A cellulose acetate filter was placed at the bottom of the cell above the frit. The extraction was conducted with ultrapure water and the extract was removed by gas flushing into glass vial as followed: cell load ing and sealing into the oven, filling up with water to the set pres sure, heating during static time, rinsing with fresh water (100% of the extraction cell volume) and purging with nitrogen. For solid liquid separation, a protein precipitation was performed by pH modification to 4.5 with HCl 1 N (isoelectric point of soybean pro teins) before centrifugation at 6000*g* for 10 min. The supernatant was filtered through a 0.22 μm cellulose acetate membrane filter.

2.4. HPLC analysis

The amount of extracted isoflavones was determined by HPLC UV analysis. A 20 μl sample was injected into the chromatographic column C12 reverse phase Phenomenex Synergi Max 150 mm × 3 mm 4 μm 80A. The mobile phase was composed of 0.1% formic acid in water (A) and acetonitrile (B). The elution was performed with a 60 min multi step gradient starting with 5 min at 10% B; 5 min to 38 min from 10% to 29% of B; 38 min to 48 min from 29% to 35% of B; 35% to 10% until 55 min followed by 5 min at 10% B. The eluent flow rate was 0.8 ml/min and UV absorbance was measured at 260 nm.

2.5. Experimental design

To optimize the pressurized water extraction of isoflavones, a Doehlert experimental design with three levels: temperature, sta tic time extraction and solid liquid ratio (quantity of sample intro duced into the extractor cell for a fixed volume delivered), was conducted. Total isoflavone aglycons, genistein, daidzein, glycitein and their derivatives were introduced in the quadratic response surface model as Eq. (1).

$$Y \quad a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_{12}X_1X_2 + a_{13}X_1X_3 + a_{23}X_2X_3 + a_{11}X_1^2 + a_{22}X_2^2 + a_{33}X_3^2 \qquad (1)$$

where Y is the model response expressed in percentage, X_i is the coded experimental level of the variables and a_i, a_{ik} and a_{ii} are coef ficients respectively for linear, interaction and quadratic effects. Temperature ranged from 40 to 200 °C, static contact time varied from 2 to 14 min and an amount of 0.35 g to 1.65 g of soybean material was introduced into the cell corresponding to a solid/liquid ratio from approximately 1 7% according to the recovery volume in the extract (25 ml).

Firstly, the response Y corresponding to the isoflavone extrac tion yield was calculated as follows:

$$IF\ Extraction\ Yield \quad \frac{m_{IF_{(ext\ ASE)}}}{initial\ m_{IF_{(ext\ Murphy)}}} \qquad (2)$$

where $m_{IF}(ext\ ASE)$ is the isoflavone (IF) quantity recovered in the water extract after ASE extraction, protein precipitation, and cen trifugation. The *initial* $m_{IF}(ext\ Murphy)$ is the initial quantity of iso flavones determined by the previously described conventional extraction. This calculation permits to compare ASE extraction method with previously reported method. All isoflavone quantities are expressed in aglycon equivalent to take into account the molec ular mass differencies between malonyl, glucoside and aglycon forms (Collison, 2008).

To study the influence of the pressurized water extraction on soybean proteins, the second response Y used for the Doehlert experimental design was the dry matter yield. This corresponded to the amount of solubilized material recovered in the supernatant after precipitation of the pressurized water extract (at pH4.5 and after centrifugation at 6000g for 10 min) and represented hydro lyzed proteins. This was calculated as follows:

$$\text{Solubilized material} \quad \frac{\frac{m_{(ext\ ASE)}}{V_{(supernatant)}} \times V_{(extract)}}{m_{(initial)}} \qquad (3)$$

where $m_{(ext\ ASE)}$ is the dry matter recovered in the supernatant, $m_{(initial)}$ is the quantity of dry material introduced into the cell.

3. Results

3.1. Isoflavone profile analysis from raw materials

SPI and SF were first characterized by aqueous acidified acetoni trile extraction (conventional method) and gave respectively 160.6 mg and 110.8 mg of total isoflavones on 100 g of dry product (Table 1.) with an isoflavone profile in accordance with the litera ture (Murphy et al., 1999).

The malonyl forms represent approximately 72% (w/w) of total flour isoflavones and glucosides 27.7%, while the other forms: acet yls and aglycons were present in trace amounts. From the Soybean Protein Isolate the amounts differed with 21.4% of aglycon, 38.7% of glucoside and 30.3% of malonyl isoflavone. The isolation process for soybean proteins has a strong influence on the isoflavone pro file with a decreasing amount of malonyl forms toward an increas ing amount of glucosides or aglycons (Villares, Rostagno, García Lafuente, Guillamón, & Martínez, 2010) which explains the differ ences between the isoflavone profiles and raw materials used in this study.

3.2. Experimental design

For the experimental design on water extraction by the ASE sys tem, coded variables were chosen according to the Doehlert exper imental design, the response Y of the model was total isoflavone extracted (Eq. (2)) expressed in equivalent aglycons (Table 2).

The isoflavone extraction yield determined with 15 experi ments ranged from 22.1 86.7% for SF extraction and 11.2 50.8% for SPI extraction. Predicted responses were close to measured responses and the model gave R squared value at 0.93 for SF and 0.92. Fisher test confirmed statistical significance with 5% level of confidence for the model. All coefficients for independent variables in the isoflavone extraction yield model were also tested with Stu dent's *t* test at 5% significance level (Table 3). The amount intro duced is the only significant variable in terms of 5% significance (Table 2) for both materials with high and low protein content.

Table 1
Isoflavone amount measured with HPLC-UV system according to Murphy.

Isoflavone	SF (mg IF/100 g)	SPI (mg IF/100 g)
Daidzein	0	13.2 ± 0.34
Glycitein	0	0
Genistein	0.70 ± 0.01	21.2 ± 0.1
Daidzein	6.64 ± 0.59	10.0 ± 0.63
Glycitine	1.42 ± 0.00	1.0 ± 0.27
Genistine	20.43 ± 0.85	51.1 ± 0.33
Acetyl-daidzine	0.44 ± 0.62	4.3 ± 0.19
Acetyl-glycitine	0	0
Acetyl-genistine	1.30 ± 0.07	11.3 ± 0.07
Malonyl-daidzine	29.22 ± 1.90	11.1 ± 0.72
Malonyl-glycitine	2.87 ± 0.05	0
Malonyl-genistine	47.75 ± 2.80	37.5 ± 0.59
Total	110.78 ± 5.55	160.62 ± 2.37

m IF expressed on dry basis in aglycon equivalent, standard deviation based on three extractions.

Table 2
Measured and predicted responses for the different levels of variables corresponding to the Doehlert experimental design for soybean flour and Soybean Protein Isolate extracted by ASE system with water.

Variables			Coded variables			SF		SPI	
T (°C)	Static time (min)	S/L[a] (%)	X1	X2	X3	Ym[b]	Yp	Ym	Yp
120	8	4	0	0	0	54.9	53.2	35.2	30.6
200	8	4	1	0	0	22.1	27.9	18.6	21.3
160	14.0	4	0.5	0.866	0	55.7	53.3	40.1	34.3
80	14.0	4	−0.5	0.866	0	52.4	59.5	29.7	30.9
40	8.0	4	−1	0	0	48.3	42.5	22.2	19.5
80	2.0	4	−0.5	−0.866	0	52.5	54.9	28.8	34.6
160	2.0	4	0.5	−0.866	0	53.5	46.4	34.1	32.9
160	10.0	7	0.5	0.289	0.816	30.3	27.0	11.9	14.9
80	10.0	7	−0.5	0.289	0.816	42.2	40.9	11.7	13.3
120	4.0	7	0	−0.577	0.816	32.7	37.4	11.2	6.6
160	6.0	1	0.5	−0.289	−0.816	71.3	72.6	49.8	48.1
80	6.0	1	−0.5	−0.289	−0.816	70.0	73.3	50.8	47.9
120	12.0	1	0	0.577	−0.816	86.7	82.1	34.8	39.4
120	8.0	4	0	0	0	54.1	53.2	29.3	30.6
120	8.0	4	0	0	0	50.4	53.2	27.4	30.6

SF for soybean flour and SPI for Soybean Protein Isolate.
[a] S/L for Solid liquid ratio.
[b] Ym = measured responses and Yp = predicted responses (% of IF mass extracted by IF mass introduced).

3.3. Surface response methodology

The surface response for isoflavone extraction is represented (Fig. 1) with the influence of the three variables temperature (T), solid liquid ratio (S/L), static extraction time (ST). A slight temper ature influence is noticed with the quadratic coefficient that causes

Table 3
Regression coefficients of the quadratic regression model and extraction yields with corresponding optimum parameters.

Coefficient	Values			
	Y = IF extraction yield**		Y = Solubilized material***	
	SF	SPI	SF	SPI
a0	53.15	30.61	19.94	22.34
a1	−7.33	0.93	11.54	20.41*
a2	3.31	−0.66	2.38	4.72
a3	−25.06*	−20.53*	−3.68	−5.04
a12	1.33	2.94	4.52	3.45
a13	−8.59	−0.21	−2.77	21.05
a23	−0.26	17.42	6.90	13.94
a11	−17.96*	−10.22	12.86	17.55*
a22	6.49	6.82	5.37	2.59
a33	6.46	−2.54	0.30	4.24
R square	0.93	0.92	0.98	0.93
F (F_{table} = 19.16)	14.37	3.00	34.59	7.76
Optimum parameters				
Ratio S/L (%)	1	1	–	7
Temperature (°C)	122	114	–	200
Time (min)	14	2	–	14
Predicted extraction yield	85.8%	63.7%	–	95.0%
Measured extraction yield	85.1 ± 4.5%	61.0 ± 0.7%	–	92.0 ± 2.1%

* Significant value at 5% level (p < 5%) with Student test.
** Measured by Eq. (2) and standard error based on three ASE extractions.
*** Measured by Eq. (3).

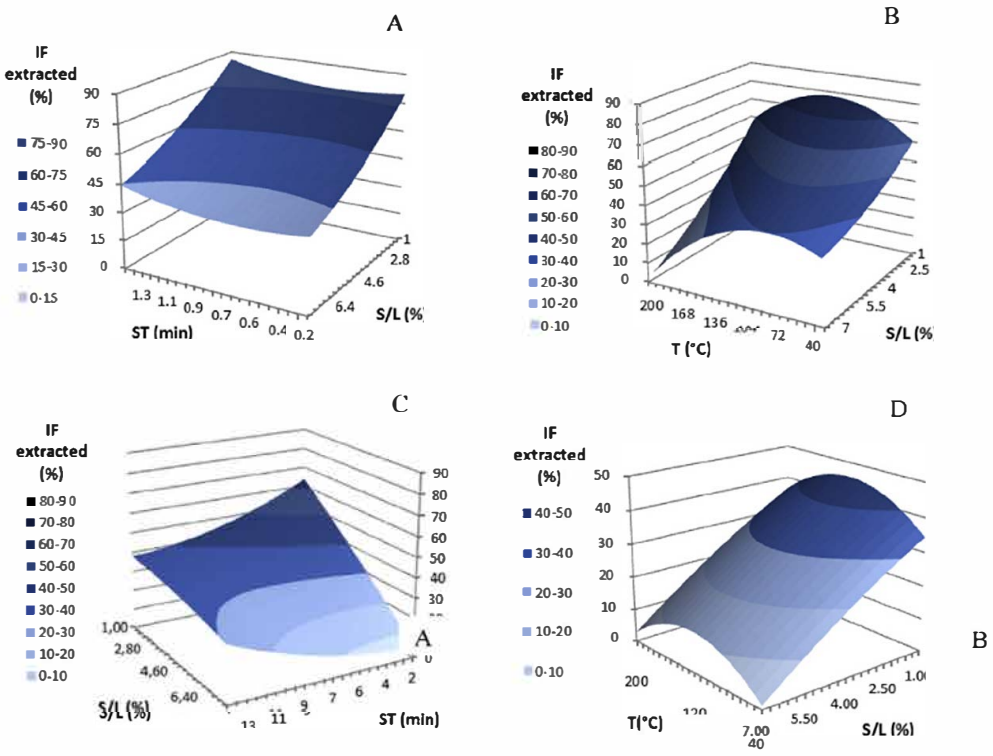

Fig. 1. Doehlert surface responses for the influence of introduced amount, static time and temperature on isoflavone extraction yield from SF (A and B) and SPI (C and D). The temperature is fixed at 120 °C for A and C response surfaces and the extraction time was fixed at 8 min for B and D, corresponding to central values given by the Doloerth experimental design.

the curve of the response. The optimum extraction parameters give an extraction yield of 63.7% for SPI and a high extraction yield of 85.8% for SF. The model was confirmed by testing optimal conditions at 120 °C, 14 min, 0.35 g and 114 °C, 2 min, 0.35 g giving respectively 61.0% and 85.1%. Three extractions were performed under optimal conditions determined by the surface response and the results correspond well with the predicted optimum yield (Table 3).

The extraction yield was very different from previous works, which gave 23.6% and 18.2% isoflavone extraction yield with water alone (Jung et al., 2008; Luthria et al., 2007; Rostagno et al., 2004). This strong variation compared to our results (85% concerning flour extraction) could be explained first of all by the study approach, whose optimization was set by solvents, water was used solely as a control. Furthermore, their selected temperature and pressure at 60 °C and 1000 psi were probably not enough to reach high recoveries when water was used as a solvent. Moreover, these studies were set for the purpose of analysis, the authors proposed lower temperatures in order to maintain the initial distribution of isoflavones due to malonyl and glucoside forms being transformed at high temperatures. Another reason for this difference could be the presence of a post treatment in this study, corresponding to the protein precipitation step, with HCl addition, in order to facilitate the solid liquid separation by centrifugation. Without this post treatment step, the extract could not be filtered due to the high protein content.

According to previous works conducted in ASE systems, the solid liquid ratio (amount introduced into the cell extractor) must be as low as possible, a high content of introduced soybean material inhibited isoflavone extraction: 0.1 g to 0.5 g of raw material was consequently used (Rostagno et al., 2004) for a recovered volume of approximately 22 ml. In our present study the lowest amount introduced, 0.35 g (S/L 1%) showed the best results. In autoclave systems, isoflavone recovery has been shown to have more efficiency than previously cited studies in ASE (Li Hsun, Ya Chuan, & Chang, 2007), which gave 60% yield at 44 bars and 110 °C to 120 °C, considering only aglycone and glucoside isoflavones from defatted soybean flour.

3.4. Influence on protein matrix

Since protein aggregation and disintegration by hydrolysis, occurs in high pressure systems, (Pourali et al., 2009; Sunphorka et al., 2012) the protein content in the extraction cell is a crucial parameter. During extraction in a pressurized system, protein aggregation is explained by the strong effect of the amount of raw material introduced into the cell. When sand was not introduced among the material, the aggregation was so strong that it could obstruct the solvent flow. Therefore, protein dispersion into the stainless steel cell, with the sand, appears to be necessary in the ASE system.

To follow the solubilization of the material, the value of the total solids recovered in the supernatant after precipitation and centrifugation of the extract was assayed as response of the quadratic model used for Doloerth experimental design (Eq. (3)). This model was only validated from SPI by the Fisher test with a confidence level of 5%. According to the Student test, temperature is a significant parameter, with a confidence level of 5% (Table 3).

Despite the precipitation of proteins at pH 4.5 (isoelectric point of native soybean proteins), a high amount remains solubilized, showing a high hydrolysis of the protein matrix by the pressurized treatment, although static time is short (2 14 min). The model response surfaces are presented in Fig. 2. For low solid liquid ratios, temperature and static time have little effect on the solubilization. When a larger quantity of material is introduced in the extraction cell with a solid/liquid ratio of 7%, these parameters have more impact on the solubilization and hence on the hydrolysis of proteins. Accordingly, it appears that the most significant protein concentration in the cell (mixture with sand) is more

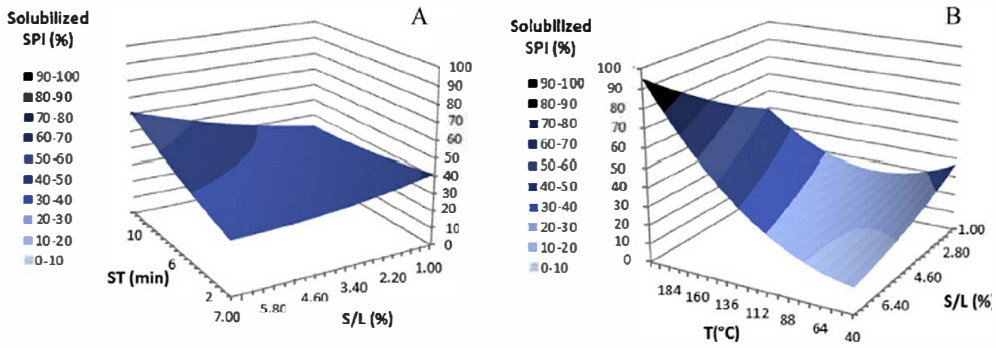

Fig. 2. Doehlert surface responses for the influence of introduced amount, static time and temperature on SPI solubilization. The temperature is fixed at 120 °C for A the extraction time is fixed at 14 min for B.

hydrolyzed than what has already been shown in other protein materials (Sunphorka et al., 2012).

From 40 °C to 200 °C, a strong increase of the solubilized material occurred, in particular for the Soybean Protein Isolate, which provides 95.0% of solubilized material at 200 °C (on dry basis) due to the protein hydrolysis which was more significant with SPI. At this temperature, total isoflavone extraction does not seem to be correlated with the total raw material solubilization. The kinetics of protein hydrolysis becomes a limiting factor in larger processed quantities which was already shown on rice bran proteins extracted by subcritical water (Sunphorka et al., 2012).

3.5. Influence of temperature on isoflavone profile and extraction yield

According to the Murphy extraction result (Table 1), the initial quantities of isoflavone from 0.35 g of SF were 7, 281, 18 and

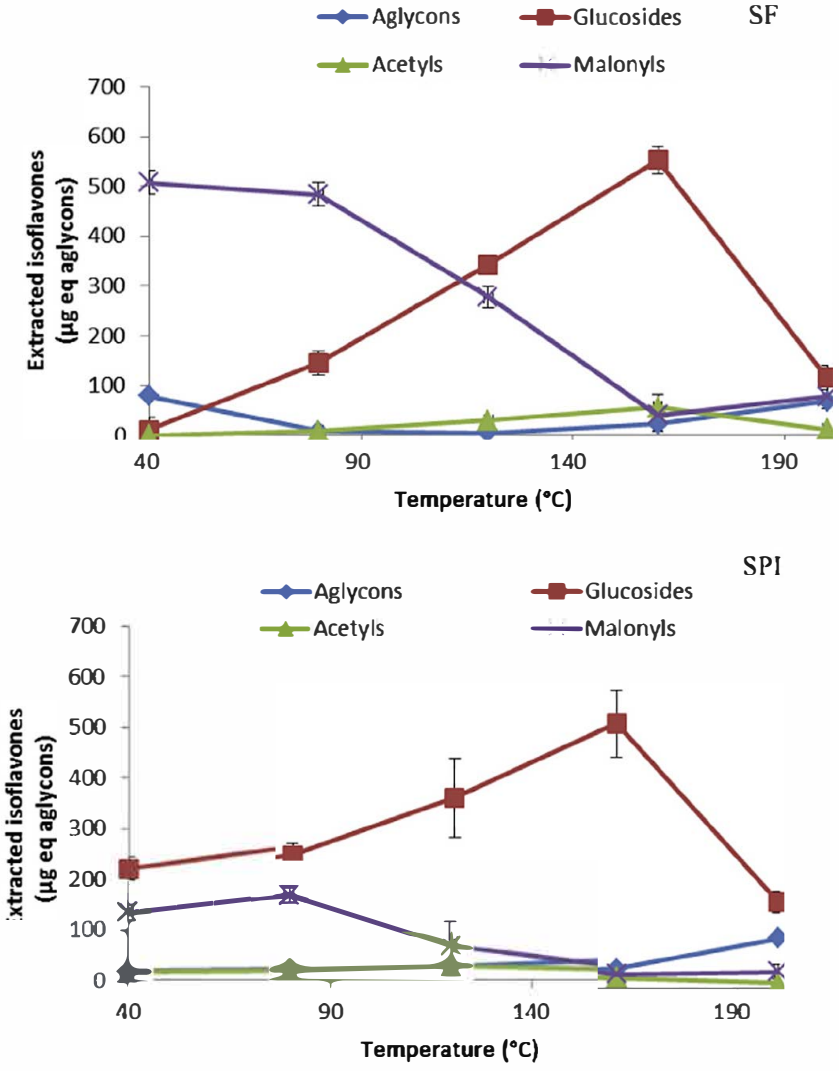

Fig. 3. Influence of temperature on the isoflavone structures for the Soybean Protein Isolate (SPI) and soybean flour (SF) the proportion of the four isoflavone types, all amounts are expressed in aglycon equivalent. The amounts of extracted isoflavones were calculated by the mean of three different extractions. Vertical bars represent the standard deviation.

915 μg of aglycones, glucosides, acetyls, and malonyls. From 0.35 g of SPI these amounts were 359, 655, 147 and 560 μg. Despite this temperature was not a significant parameter for total isoflavone recovery, a significant effect on the structure of isoflavone derivatives was demonstrated from the analysis of recovered extract with an increasing temperature. The extraction of aglycones is not significant, even from the SPI, which contains 21% of aglycon isoflavones (Fig 3). Due to the weak polarity of these coumpounds, their extractability is lower with a polar solvent like water. This fact could also explain the total extraction differencies between materials. The proportion of aglycon isoflavones was 21% and less than 1% in the SPI and SF respectively. The difference between isoflavone extraction yields from both materials was not clearly correlated to protein hydrolysis.

At low temperatures (80 °C), 55% of the malonyl forms were extracted from SF and only 23% from SPI, although SPI contains less malonyl isoflavone. This lower amount of malonyl isoflavones would cause a higher extraction yield. This indicates retention of malonyl compounds in protein matrix, which is more prevalent in SPI. In another study, during protein isolation and precipitation these malonyl forms displayed specific interactions with soybean proteins, leading to their retention on SPI (Speroni, Milesi, & Añón, 2010). Glucoside extraction yields were 58% and 42% from SF and SPI respectively, possibly due to their initial concentration, which is greater in flour.

From 80 °C to 160 °C (Fig. 3) an increasing quantity of glucosides appeared which correlated with a decrease in malonyl content (data expressed in aglycones equivalent). This phenomenon showed the elimination of malonyl groups in this temperature range. The instability of malonyl glucosides in pressurized systems was shown in ASE as studied by Rostagno et al. (2004) and Jung et al. (2008), Rostagno, Araújo, and Sandi (2002) and Rostagno et al. (2004). They showed the conversion of malonyl forms into glucoside forms when exceeding 60 °C and the conversion of glucosides into aglycones when exceeding 160 °C, consistent with our results. At 200 °C, glucoside forms are converted into aglycones but these forms were not well extracted by 100% water (Fig. 3), unlike Rostagno et al. (2004) who performed extraction with organic solvent which permitted the extraction of less polar isoflavones like aglycons.

The effectiveness of pressurized water in extracting isoflavones is strongly dependent on the isoflavone profile especially the proportion of aglycon forms. To optimize the extraction of total isoflavones from soybean material, operating temperatures have to be less than 160 °C to avoid the conversion of glucoside into aglycones. Because processed materials like SPI contain more aglycones, extraction of total isoflavones is less effective. This optimization permits to obtain an isoflavones extraction yield very close to the conventional extraction by acetonitrile. As mentioned by Murphy, the mixture of acetonitrile and acidified water is the most efficient solvent to extract all isoflavones from soybean products (Patricia A. Murphy, Barua, & Hauck, 2002).

4. Conclusion

The optimization of isoflavone extraction by pressurized water using the Doehlert experimental design was conducted from Soybean Protein Isolate and soybean flour giving high optimum yields of 65% and 87% respectively. The main factor involved in the study of extraction efficiency was the solid liquid ratio used. Optimal conditions were tested and gave extraction yields in line with the theoretical values. Elevated hydrolysis of the material occurred in the extraction cell and more particularly from the protein isolate. Consequently, with a treatment at 200 °C for 14 min, dry matter was solubilized up to 95%. Pre aggregation and hydrolysis were not the primary reason for the observed difference between the isoflavone extraction yield from soybean flour and Soybean Protein Isolate. The presence of aglycon isoflavones within the Soybean Protein Isolate induced a lower extraction yield of total isoflavones because their chemical structures are less hydrophilic. This technique could be used as a treatment of vegetable protein in their solubilization. Further studies should be conducted to clarify the understanding of the PW impact on the protein structure, molecular weight and chemical properties.

The study of isoflavones based on temperature profiles showed that malonyl forms are transformed into glucoside forms at between 80 °C and 160 °C for both materials. With temperatures exceeding these, the glucoside forms deteriorate and the total isoflavone extraction yield decreases. The glucosides are converted into aglycones, and these forms are extracted with much lower yields. These forms are poorly extracted from the protein isolate, which contain around 20%. Low temperatures during the extraction of malonyl forms prevent their degradation and reveal retention by the isolated proteins unlike soybean flour material. Furthermore, this could explain the lower isoflavone yield derived from isolated proteins. These results show that the extraction of isoflavones by pressurized water is limited to most polar conjugated forms (glucosides, malonyls) and by the proportion of malonyl forms, which are more preserved on protein material. In addition to the solid/liquid ratio, the extraction efficiency of pressurized water is highly dependent on the distribution of isoflavones.

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