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Aqueous micellar two-phase system as an alternative method to selectively remove soy antinutritional factors

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ABSTRACT

In this work, different antinutritional factors (trypsin inhibitors, isoflavones and raffinose family oligosaccharides) were selectively removed from soy flour by using aqueous micellar two-phase systems (AMTPS). The effects of independent variables including temperature (30–60 °C), time (10–40 min) and solid to liquid ratio (0.025–0.050 g/L) on the extraction of each antinutritional factor were analyzed using a full factorial design. As general tendency, temperature and time were the most significant parameters ($p < 0.05$). The best condition for the selective recovery (97% of isoflavones at top phase, and more than 50% of the rest of ANFs at bottom phase) were 5 g/L of Genapol X-080, 0.2 moL/L of sodium citrate pH 5.00, 30 °C, 40 min and 0.050 g/L. Besides, *in vitro* gastrointestinal digestions assays demonstrated that the treated soy flour improved its protein digestibility. The findings of this work represent the introduction of a novel methodology to selectively remove soy antinutritional factors.

1. Introduction

At present, soybeans (*Glycine max (L) Merril*) represent one of the most important sources of nutritional proteins (Yu, Yuan, Fu, & Zhu, 2016). It is estimated that 60% of total processed food includes ingredients derived from soy (Praveen Kumar & Mulimani, 2010). Nevertheless, a soy-based diet can present some disadvantages due to the presence of certain components known as antinutritional factors (ANFs) (Becker-Ritt, Mulinari, Vasconcelos, & Carlini, 2004). These compounds, such as oligosaccharides, phytoestrogens and protease inhibitors, can negatively affect animals and humans health when consumed frequently (Vagadia, Vanga, & Raghavan, 2017; Yu et al., 2016). Trypsin inhibitors (TI) are considered as the major soy ANFs (Sousa et al., 2015). High levels of these proteins could inhibit digestive proteases, thus affecting protein digestibility, and causing certain diseases, such as pancreatic hypertrophy (Vagadia et al., 2017). Examples of less harmful ANFs are raffinose and stachyose (RFOs), which have been associated with nutrient digestibility reduction, flatulence and abdominal discomfort (Dersjant-Li & Peisker, 2010; Praveen Kumar & Mulimani, 2010). Additionally, soy isoflavones (IF), also known as phytoestrogens, can exhibit undesirable physiological effects on human metabolism, principally at childhood (Portman, Navarro, Bruce, & Lampe, 2016).

A plethora of processing methods, such as soaking, cooking, toasting and chemical treatments, have already been explored in order to inactive/reduce soy ANFs (Akbarian et al., 2014; Dersjant-Li & Peisker, 2010). Heating seems to be the most suitable processing method to reduce TI activity. Trypsin inhibitory effect have been reduced up to 85% (i.e. remaing only 15% of initial TI activity) using different heating protocols such as oven dry heat and salt-bed roasting (Coscueta et al., 2017). However, extreme working conditions such as high temperatures can compromise the availability of other components. Thus, alternative methodologies, such as radiation and oxidation, are being evaluated (Vagadia et al., 2017). With regard to RFOs, solvent extraction and enzymatic degradation are the most common means to eliminate them (Dersjant-Li & Peisker, 2010; Praveen Kumar & Mulimani, 2010). Respecting to IF reduction, solvent extractions in aqueous and organic media represent the most used methodologies (Jankowiak, Kantzas, Boom, & Van Der Goot, 2014; Sun, Li, & Wang, 2011).

Although most of the previously mentioned ANFs are known for

Abbreviations used: AMTPS, aqueous micellar two-phase system; ANFs, antinutritional factors; BAPNA, α-N-benzoyl-DL-arginine-p-nitroanilide; CI, confidence interval; D, deactivated soy flour; GX, Genapol X-080; IF, isoflavones; IGD, *in vitro* gastrointestinal digestion; K_r, partition coefficient; ND, non deactivated soy flour; RA, relative units; RFOs, raffinose family oligosaccharides; TI, trypsin inhibitors; TIU, trypsin inhibitors units; S_T, selectivity at top phase

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their adverse effects, it is true that many of them also have beneficial effects on health (Thompson, 1993). For example, TI is known to be involved in many biological functions, such as blood coagulation, platelet aggregation, anti-carcinogenesis and granulo-cytopoietic activity (da Silva Bezerra et al., 2015). Besides, IF consumption has been associated with reduced menopause symptoms, reduced incidences of hyperglycemia and improved bone quality (Ahn & Park, 2017; Cordisco, Haidar, Coscueta, Nerli, & Malpiedi, 2016). Thereby, the development of strategies for the selective and non-destructive removal of soy ANFs represents a research area of great interest.

Aqueous micellar two-phase systems (AMTPS) represent an attractive tool to selectively extract soy ANFs. This methodology, which is based on solid-liquid and liquid-liquid extraction, depends on the ability of some surfactants to form two immiscible aqueous phases, a micelle-rich phase and a micelle-poor phase, over certain temperature defined as cloud point (Gu & Galera-Gómez, 1995). Thereby, the physicochemical differences between both phases allow the separation of biomolecules present in a mixture (Bordier, 1981). At present, this technique has gained relevance as an eco-friendly methodology to purify a wide variety of molecules such as enzymes, antibodies, antibiotics and polyphenols (Sharma, Kori, & Parmar, 2015).

Preliminary works carried out by our research group have already demonstrated that IF can be successfully purified at the micelle-rich phase of AMTPSs of Triton X-114 and sodium tartrate (Cordisco et al., 2016). Under optimal working conditions, IF were purified with a recovery percentage of 93 and a purification factor of almost 10. However, other ANFs have not been analyzed.

Thus, in this context, the main aim of this work was to evaluate for the first time the feasibility of using AMTPS to selectively extract different antinutritional factors (raffinose, trypsin inhibitor and isoflavones) from soy flour. Genapol X-080 was selected as micelle-forming surfactant since its use was approved by the Food and Drug Administration (FDA). Protein availability of the treated soy flour was also evaluated.

2. Materials and methods

2.1. Materials

Defatted soybean flour, both deactivated (D, treated with oven dry heat at 80 °C for 1 h) and non-deactivated (ND) samples, were obtained from the food processing company Molinos Río de la Plata SA (San Lorenzo, Argentina). Trypsin (bovine), pepsin (from porcine gastric mucosa), pancreatin (from porcine pancreas), bile (from bovine bile), α-N-benzoyl-DL-arginine-p-nitroanilide (BAPNA) and Tris buffer were purchased from Sigma-Aldrich (St. Louis, USA) and used without further purification. The non-ionic surfactant polyethylene glycol monoalkyl ether (Genapol) X-080 (GX), citric acid and bicinchoninic acid (BCA) were supplied by Sigma–Aldrich (St. Louis, USA) and used as received. All the other reagents were of analytical grade and used without further purification.

2.2. Experimental design

The extraction of soy ANFs was performed with the aid of a 2^3 –full factorial design with three repetitions at the central point (Table 1). Temperature (X_1, °C), time (X_2, min) and solid to liquid ratio (X_3, g/L) were the independent variables. The recovered amount of each ANFs constituted the analyzed responses. Selectivity (S) at top or bottom phases and partition coefficients (K_r) were also evaluated (equations described at section 2.8).

2.3. Liquid-liquid extraction assays

ANFs extraction with aqueous micellar two-phase systems was performed by using 50 g/L Genapol X-080 (GX) in sodium citrate (NaCit) 0.2 moL/L, pH 5.00. Notice that in this type of AMTPS the top phase is enriched in surfactant micelles while the bottom phase presents scarce amount of these aggregates (Cordisco, Haidar, Goñi, Nerli, & Malpiedi, 2015).

Table 1
Full factorial design 2^n (n: numbers of independent variables) for the study of soy flour antinutritional factors extraction by using aqueous micellar two-phase systems, prepared with 5 g/L of Genapol X-080 and 0.2 moL/L of sodium citrate pH 5.00. Independent variables: X_1 = temperature (°C); ×2 = time (min); X_3 = solid to liquid ratio (g/L).

Run	Coded and real independent variables					
	X_1	X_2	X_3	$X_{1\ (°C)}$	$X_{2\ (min)}$	$X_{3\ (g/L)}$
1	−1	−1	−1	30	10	0.050
2	+1	−1	−1	60	10	0.050
3	−1	+1	−1	30	40	0.050
4	+1	+1	−1	60	40	0.050
5	−1	−1	1	30	10	0.025
6	+1	−1	1	60	10	0.025
7	−1	+1	1	30	40	0.025
8	+1	+1	1	60	40	0.025
9[a]	0	0	0	45	25	0.033
10[a]	0	0	0	45	25	0.033
11[a]	0	0	0	45	25	0.033

[a] Central points.

The preparation of the studied systems was carried out by weighing (analytical balance Pioneer™ Plus, Ohaus, Parsippany, USA) into graduated glass tubes each system component: ND soy flour (0.100, 0.150 or 0.200 g, according to the run number of Table 1), GX (0.250 g of pure surfactant) and sodium citrate buffer 0.2 moL/L, pH 5.00 (until reaching a final mass of 5.000 g). The prepared systems were then mixed at 30 rpm for 1 h at room temperature using a tube rotator apparatus (Bioelec®, Santa Fe, Argentina). After that, the systems were incubated in a water bath (Tecnodalvo, Santa Fe, Argentina) at the different conditions presented in Table 1. At the end of the incubation step, both phases were conveniently separated by centrifuging at 1970 × g for 10 min (refrigerated benchtop centrifuge, Sigma Laborzentrifugen 3–18 KS, Osterode, Germany) at the same temperature of incubation. Finally, samples from top and bottom phases were taken for the determination of partition coefficients and recoveries of ANFs (IT, IF and RFOs). The treated soybean flour, which was totally recovered at the bottom of the test tube, was dried and stored for further analysis.

2.4. ANFs extraction with reference methods

2.4.1. Trypsin inhibitors

The extraction of TI was performed by following the AOCS official method (AOCS, 2009; Coscueta et al., 2017). The obtained supernatant was used for determination of TI activity.

2.4.2. Isoflavones

IF extraction was performed by suspending 1.000 g of ND soy flour into 50.0 mL of extracting solution (pure methanol/water in 4:1 mL:mL). The suspension was homogenized at 30 rpm (Age magnetic stirrer, Velp Científica, Usmate, Italy) for 3 h at 35 ± 0.1 °C (thermostated incubator, San Jor, San Andrés, Argentina). After centrifuging at 2460 × g for 15 min at room temperature (refrigerated benchtop centrifuge, Sigma Laborzentrifugen 3–18 KS, Osterode, Germany), supernatant was used for isoflavone quantification.

2.4.3. Raffinose family oligosaccharides

The procedure consisted in adding 1.000 g of ND soy flour into 50.0 mL of ethanol/water mixture (Kumar Dixit, Kumar, Rani, Manjaya, & Bhatnagar, 2011). The resulting suspension was homogenized at