Food Chemistry 271 (2019) 274–283

Contents lists available at ScienceDirect

Food Chemistry

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

Parameters of the fermentation of soybean flour by *Monascus purpureus* or *Aspergillus oryzae* on the production of bioactive compounds and antioxidant activity

Cíntia Ladeira Handa[a], Fernando Sanches de Lima[a], Marcela Fernanda Geton Guelfi[a], Meg da Silva Fernandes[a], Sandra Regina Georgetti[b], Elza Iouko Ida[a,*]

[a] *Universidade Estadual de Londrina, Departamento de Ciência e Tecnologia de Alimentos, 86051-990 Londrina, PR, Brazil*
[b] *Universidade Estadual de Londrina, Departamento de Ciências Farmacêuticas, Brazil*

ARTICLE INFO

Chemical compounds studied in this article:
Daidzein (PubChem CID: 5281708)
Daidzin (PubChem CID: 107971)
6″-O-Acetyldaidzin (PubChem CID: 156155)
6″-O-Malonyldaidzin (PubChem CID: 9913968)
Genistein (PubChem CID: 5280961)
Genistin (PubChem CID: 5281377)
6″-O-Acetylgenistin (PubChem CID: 5315831)
6″-O-Malonylgenistin (PubChem CID: 53398685)
Glycitein (PubChem CID: 5317750)
Glycitin (PubChem CID: 187808)
6″-O-Acetylglycitin (PubChem CID: 10228095)
6″-O-Malonylglycitin (PubChem CID: 23724657).

Keywords:
Solid-state fermentation
Initial pH of the substrate
Substrate-to-water ratio
Incubation temperature
Antioxidant activity

ABSTRACT

The objective of this work was to evaluate the effects the solid-state fermentation parameters of defatted soybean flour (DSF) by *Monascus purpureus* or *Aspergillus oryzae* on the bioactive compounds. Central composite rotatable design, multi-response optimization, and Pearson's correlation were used. The fermentation parameters as initial pH (X_1), DSF-to-water ratio (X_2), and incubation temperature (X_3) were taken as independent variables. The function responses were isoflavone content, total phenolic content (TPC), and antioxidant activity. All fermentation parameters affected the isoflavone content when fermented by *Monascus purpureus*, whereas the TPC or antioxidant activities remained almost unchanged. For the fermentation *by Aspergillus oryzae*, all the function responses were influenced by X_2 and X_3 and were independent of the X_1. Estimated optimum conditions were found as $x_1 = 6.0$, $x_2 = 1{:}1$, and $x_3 = 30\,^\circ\mathrm{C}$ for both fungi. Achieving suitable fermentation parameters is essential to increase bioactive compounds in the DSF that makes it promising for food industrial applications.

1. Introduction

Soybeans are the most important oilseed in the world. A large quantity of defatted soybean flour (DSF) is obtained from the oil extraction process. DSF has a high protein content and typically consists of 50% proteins, 40% carbohydrates, 7.5% water, and other minor components such as saponins, phenolic compounds, isoflavones, and essential amino acids (Chen, Lin, Rao, & Zeng, 2013; Hassaan, Soltan, & Abdel-Moez, 2015; Muttakin, Kim, & Lee, 2015). A large amount of the defatted soybean flour is used in animal feed. However, it can be applied to the food industry as an ingredient (Muttakin, et al., 2015; Villalobos et al., 2016). The isoflavones are the main phenolic compounds in soybean and are distributed in aglycone forms (daidzein, glycitein, and genistein) and their respective acetyl-glucoside, malonyl-glucoside, and glucoside forms (glycosylated forms) totaling 12 isoflavones. It is emphasized that the aglycone forms have greater availability and antioxidant activity than glycosylated forms (Moras, Rey, Vilarem, & Pontalier, 2017; Xu & Chang, 2008).

Soybean fermentation has been used to develop specific foods and as a strategy for enriching soy-based products containing phenolic antioxidants, which are associated with consumer good health and well-being (McCue & Shetty, 2003). In the fermentation process by microorganisms, antioxidant phenolic compounds are either produced via secondary metabolic pathways or released from the substrate by

* Corresponding author.
E-mail address: elida@uel.br (E.I. Ida).

https://doi.org/10.1016/j.foodchem.2018.07.188
Received 20 October 2017; Received in revised form 22 April 2018; Accepted 25 July 2018
Available online 26 July 2018

Table 1
Central composite rotatable design and response variables expressed in the defatted soybean flour fermented by *Monascus purpureus* (DSFF-Mp) and defatted soybean flour fermented by *Aspergillus oryzae* (DSFF-Ao), X_1 (initial pH of DSF); X_2 (DSF-water ratio, w/v); X_3 (°C incubation).

Independent variables coded and uncoded				Response-functions of DSFF- Mp								Response-functions of DSFF- Ao							
Run	x_1 (X_1)	x_2 (X_2)	x_3 (X_3)	MGLU Mp	AGLU Mp	GLU Mp	AGLY Mp	TPC Mp	DPPH Mp	FRAP Mp	ABTS Mp	MGLU Ao	AGLU Ao	GLU Ao	AGLY Ao	TPC Ao	DPPH Ao	FRAP Ao	ABTS Ao
1	−1 (5.5)	− 1 (1:0.5)	−1 (20)	0.25	0.59	3.13	0.76	2.15	0.69	13.85	47.4	0.24	0.67	3.34	0.78	2.09	0.22	16.04	59.5
2	−1 (5.5)	−1 (1:0.5)	+1 (40)	0.23	0.60	2.47	2.04	2.02	0.72	13.58	58.7	0.26	0.59	2.95	0.81	2.02	0.12	11.37	56.58
3	−1 (5.5)	+1 (1:1.5)	−1 (20)	0.43	0.46	3.15	1.28	2.42	0.7	14.96	55.53	0.2	0.63	3.65	0.93	2.42	0.21	17.11	59.43
4	−1 (5.5)	+1 (1:1.5)	+1 (40)	0.28	0.36	0.72	4.57	2.45	0.69	14.87	54.95	0.22	0.54	2.39	1.87	2.45	0.24	23.62	62.73
5	+1 (6.5)	−1 (1:0.5)	−1 (20)	0.35	0.59	3.44	0.94	2.32	0.63	14.66	60.03	0.36	0.51	3.21	0.72	2.42	0.25	12.5	54.98
6	+1 (6.5)	−1 (1:0.5)	+1 (40)	0.24	0.52	2.19	2.12	2.06	0.69	13.57	50.88	0.14	0.5	3.15	0.9	2.07	0.17	15.99	51.95
7	+1 (6.5)	+1 (1:1.5)	−1 (20)	0.32	0.48	3.45	1.19	2.43	0.65	14.42	65.28	0.26	0.6	3.77	0.9	2.43	0.28	15.85	64.97
8	+1 (6.5)	+1 (1:1.5)	+1 (40)	0.32	0.55	1.19	3.48	2.4	0.64	14.29	53.59	0.32	0.45	2.95	1.44	2.5	0.28	18.14	59.36
9	0 (6.0)	0 (1:1)	0 (30)	0.18	0.36	1.24	3.86	2.29	0.72	15.03	64.82	0.17	0.44	2.66	1.67	3.4	0.36	25.31	78.01
10	0 (6.0)	0 (1:1)	0 (30)	0.16	0.41	1.55	3.59	2.32	0.69	14.38	56.92	0.16	0.49	2.39	1.71	3.63	0.31	27.46	83.98
11	−1.68 (5.2)	0 (1:1)	0 (30)	0.48	0.53	2.14	2.18	2.25	0.73	14.57	59.67	0.13	0.56	2.05	2.01	3.8	0.36	23.46	89.28
12	+1.68 (6.8)	0 (1:1)	0 (30)	0.29	0.50	2.73	1.86	2.14	0.72	14.81	57.76	0.12	0.49	2.67	1.68	4.47	0.33	29.55	79.5
13	0 (6.0)	−1.68 (1:0.2)	0 (30)	0.31	0.78	3.27	0.73	2.12	0.73	15.22	58.97	0.18	0.87	3.30	0.75	2.23	0.19	15.65	55.82
14	0 (6.0)	+1.68 (1:1.8)	0 (30)	0.29	0.42	1.40	3.46	2.25	0.75	15.44	61.66	0.28	0.47	1.97	1.95	4.26	0.32	25.16	100.08
15	0 (6.0)	0 (1:1)	−1.68 (13)	0.48	0.55	3.20	0.85	2.03	0.74	13.25	63.23	0.33	0.66	3.63	0.88	2.23	0.00	15.1	64.3
16	0 (6.0)	0 (1:1)	+1.68 (47)	0.18	0.53	2.70	1.87	1.91	0.72	12.85	59.09	0.22	0.6	3.32	1.18	2.08	0.00	14.4	58.51
17	0 (6.0)	0 (1:1)	0 (30)	0.48	0.44	1.32	3.2	1.95	0.72	14.16	58.38	0.13	0.51	2.55	1.85	3.73	0.14	26.11	79.29
18	0 (6.0)	0 (1:1)	0 (30)	0.47	0.43	1.04	3.25	2.15	0.76	14.49	61.47	0.16	0.55	2.72	1.65	3.45	0.16	24.29	83.97

MGLU = malonyl-β-glucoside isoflavones (μmol/g); AGLU = acetyl-β-glucoside isoflavones (μmol/g); GLU = β-glucoside isoflavones (μmol/g); AGLY = aglycone isoflavones (μmol/g); TPC = total phenolic content (mg GAE/g); DPPH = antioxidant activity determined by DPPH (μmol TE/g); ABTS = antioxidant activity determined by ABTS (μmol TE/g); FRAP = ferric reducing antioxidant power (μmol TE/g).
Mp = DSFF-Mp; Ao = DSFF-Ao.

enzymes produced by the microorganisms, thus improving the phenolic content and antioxidant activity (Dey, Chakraborty, Jain, Sharma, & Kuhad, 2016; Dulf, Vodnar, & Socaciu, 2016).

Solid-state fermentation (SSF) is an ancient culture method that is still used to produce enzymes and secondary metabolites; therefore, its application in the food industry is important for producing biomolecules such as organic acids, pigments, phenolic compounds, and flavour (Soccol et al., 2017). SSF has been applied to soybean products to produce isoflavones (Yaakob et al., 2011), enzymes (Handa, Couto, Vicensoti, Georgetti, & Ida, 2014; Li, Loman, Coffman, & Ju, 2017), and food bio-colour (Mhalaskar & Thorat, 2016); to increase proteins and hydrolysed amino acids content; to decrease the levels of phytic acid, trypsin inhibitors, raffinose, stachyose, and verbascose (Chen, Madl, & Vadlani, 2013; Hassaan et al., 2015); and to improve the antioxidant activity (Xiao et al., 2015).

The efficiency of SSF depends on the microorganism as well as the operational and environmental conditions, such as temperature, pH, moisture content, aeration, nutrient concentrations, and nature of the substrate (Farinas, 2015). Fungi are preferred for use in the SSF process because the culture conditions are similar to those that the microorganisms require in the natural environment (Raimbault, 1998). Among the fungi, *Aspergillus oryzae* has been used in SSF and is listed as "Generally Recognized as Safe (GRAS)". *Aspergillus oryzae* has a long history of use in the food industry due to its high proteolytic and amylolytic activities and produces traditional fermented foods (Kawauchi & Iwashita, 2014; Li et al., 2016). *Monascus purpureus* is an edible and versatile fungus, which produces secondary metabolites, mainly pigments, and has been used in SSF for centuries (Srianta, Zubaidah, Estiasih, Yamada, & Harijono, 2016). Moreover, its natural pigments that can be applied for coloration of meat, fishes, cheese, beer, bakery foods and pates (Vendruscolo et al., 2016). The evaluation of different strains, carbon sources and process parameters to optimize the production process has been previously investigated (Thomas, Larroche, & Pandey, 2013).

Temperature, moisture content, and pH are considered the main parameters that should be evaluated in SSF, as well as the interactions among these parameters (Farinas, 2015). These parameters can be optimized based on factorial design experiments and response surface methodology to identify the critical parameters and their interactions (Thomas et al., 2013). Handa et al. (2014) investigated that the effects of the initial pH of DSF, the water content and incubation temperature during SSF by *Monascus purpureus* or *Aspergillus oryzae* on in the production of β-glucosidase, however, the effect of these parameters on the production of bioactive compounds, mainly aglycone isoflavones, and the antioxidant activity has not been investigated. Therefore, the objective of this study was to evaluate the effects the solid-state fermentation parameters of defatted soybean flour by *Monascus purpureus* or *Aspergillus oryzae* on the bioactive compounds using a central composite rotatable design (CCRD), multi-response optimization, and Pearson's correlation.

2. Material and methods

2.1. Material

Commercial DSF was purchased from a local store in Londrina, PR, Brazil, (8.95% moisture, 1.07% lipids, 48.96% protein (N × 6.25), 5.98% ash, and 35.04% carbohydrates) and was used as a substrate for solid-state fermentation (SSF). *Aspergillus oryzae* IOC 3999/1998 (Oswaldo Cruz Foundation, Fiocruz, RJ, Brazil) and *Monascus purpureus* NRRL 1992 (GenBank: JQ614061.1, Laboratory of Biochemistry and Applied Microbiology of the Institute of Food Science and Technology of the Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil) were used in this study. Acetyl-β-glucosides (6″-O-acetyldaidzin, 6″-O-acetylgenistin and 6″-O-acetylglycitin) and malonyl-β-glucosides (6″-O-malonyldaidzin, 6″-O-malonylgenistin, and 6″-O-malonylglycitin) were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). β-glucosides (daidzin, genistin, and glycitin) and aglycones (daidzein, genistein, and glycitein) were purchased from Sigma Aldrich Co. (St. Louis, MO, EUA). 2,2-di(4-*tert*-octylphenyl)-1-picrylhydrazyl (DPPH·), 2,4,6-Tri(2-pyridyl)-S-triazine (TPTZ), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), Folin-Ciocalteu reagent, gallic acid, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma Aldrich Co. All reagents used in the analysis were of analytical grade or liquid chromatography grade.