Enzyme and High Pressure Assisted Extraction of Tricin from Rice Hull and Biological Activities of Rice Hull Extract

Chae-Young Park, Sohyun Kim, Dabeen Lee, Dong June Park¹, and Jee-Young Imm*

Department of Foods and Nutrition, Kookmin University, Seoul 02707, Korea

¹Korea Food Research Institute, Seongnam, Gyeonggi 13539, Korea

Received October 1, 2015
Revised October 23, 2015
Accepted October 27, 2015
Published online February 29, 2016

*Corresponding Author
Tel: +82-2-910-4772
Fax: +82-2-910-5249
E-mail: jyimm@kookmin.ac.kr

pISSN 1226-7708
eISSN 2092-6456

© KoSFoST and Springer 2016

Abstract Enzymatic hydrolysis was performed in conjunction with high hydrostatic pressure (HPP) treatment to extract triclin from rice hull. Enzymatic hydrolysis performed with Celluclast (0.5%, w/w) prior to HPP (500 MPa) treatment yielded maximum triclin content (32.9 mg/kg rice hull). The triclin content obtained by conventional solvent extraction and HPP treatment alone were 14.7 and 19.7 mg/kg rice hull, respectively. The rice hull extract containing triclin displayed antioxidant, anti-inflammatory, and antiadipogenic activities. The efficacy of rice hull extract obtained by either HPP or combination of enzymatic hydrolysis and HPP treatments was significantly greater than that of extract prepared by traditional solvent extraction.

Keywords: rice hull, triclin, enzymatic hydrolysis, high hydrolytic pressure, biological activities

Introduction

Rice is the most important staple diet of Asian countries. Rice hull (husk) accounts for 20% of the dry weight of harvested rice and is produced as a by product of milling process. Rice hull contains 28.6% cellulose, 28.6% hemicellulose, 24.4% lignin and 18.4% extractable matter, including phenolic compounds (1). Biological activity of rice grains (2) and bran (3) has been reported extensively; however, the biological activity of rice hull has not evaluated in detail.

Tricin (5,7,4-trihydroxy-3,5-dimethoxyflavone) is a flavonoid that is present in the outer layers of various cereal crops. In the previous studies, the health promoting effects of triclin including antioxidant activity (4), immune modulating activity (5), and cancer preventive activity (6) have been reported. In addition, the authors firstly demonstrated anti-adipogenic effect of triclin isolated in the oat hull extract (7). Moheb *et al.* (8) reported that winter wheat hull is a good source of triclin and it contained 408–772 mg triclin/kg dry weight. Tricin was also regarded as one of the major phenolic compounds (36 mM) in sugarcane juice and rice bran (5–190 mg/100 g) (4,9).

Enzyme assisted extraction is an efficient method to extract intercellular bioactive compounds from plant resources since hydrolytic enzymes facilitate release of intercellular bioactive compounds by breaking down the cell wall constituents (10). High hydrostatic pressure (HPP) extraction also improves the extraction efficiency of bioactive compounds owing to shorter extraction time and enhanced solvent permeability (11).

This study was conducted to investigate the combined effect of enzymatic hydrolysis and HPP treatment on the extraction of triclin and other polyphenols from rice hull. In addition, the effects of rice hull extract on oxidation, inflammation, and lipid accumulation were examined in an *in vitro* bioassay.

Materials and Methods

Materials Rice hull from Japonica type rice cultivar (*Oriza sativa* L.) was kindly provided by Songpo agricultural association (Ilsan, Korea). The average size of rice hull was 7 mm in length, 4 mm in width and 0.4 mm in thickness. All organic solvents including acetonitrile, and ethanol were HPLC grade and obtained from Fischer Scientific Co. (Fair Lawn, NJ, USA). Tricin was purchased from Dalton Pharma Services (Toronto, Canada). RAW 264.7 and 3T3-L1 cells were purchased from ATCC (Manassas, VA, USA). Dulbecco's Modified Eagle's Medium (DMEM), penicillin-streptomycin solution and fetal bovine serum (FBS) were purchased from WelGENE Inc. (Daegu, Korea). All other chemicals were of analytical grade and purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

Extraction of triclin from rice hull by combined treatment of enzymatic hydrolysis and HPP The rice hull was thoroughly washed with tap water and dried. For enzymatic hydrolysis, distilled water (1 L) was added to rice hull (100 g) and pH of the reaction

medium was adjusted to 5.0. The commercial food-grade enzymes (0.5%, v/w) including Celluclast (Novozymes, Bagsvaerd, Denmark), Pectinex (Novozymes), Rapidase (Novozymes), or Viscozyme (DSM Food Specialties, Delft, Netherlands) was added to the reaction medium. Enzymatic hydrolysis was carried out in a 40°C water bath for 3 h. The sample was boiled for 10 min to inactivate the enzyme. For HPP extraction, distilled water was carefully discarded and equal volume of ethanol was added to the sample. The sample was placed in a polyethylene bag and was subjected to pressure of 100-500 MPa for 6 min in an HPP system (Innoway, Beijing, China) at 20°C. After HPP treatment, the extracted material was collected by removing solvent using a rotary evaporator (Eyela, Tokyo, Japan) at 40°C. For conventional extraction (control), the sample was extracted with ethanol at 20°C for 24 h under agitation.

Tricin content determination Tricin content in rice hull extract was determined by analytical HPLC system (Jasco, Tokyo, Japan). The sample (20 mL) solubilized in methanol was injected into a C18 column (5 mM, 150x4.6 mm; Phenomenex, Torrance, CA, USA) and eluted with a linear gradient of 0-40% acetonitrile in 10 mM formic acid over 40 min at a flow rate of 0.1 mL/min. Tricin was detected at 340 nm and tricin content was calculated from the standard curve obtained with pure tricin standard (Dalton Pharma Services).

Total phenolic content determination The total phenolic content of rice hull extract was determined by the method of Singleton *et al.* (12). Briefly, Folin-Ciocalteu reagent and sodium carbonate were added to aliquots of samples and the mixtures were placed in a 40°C water bath for 20 min. The absorbance was measured at 740nm using a spectrophotometer (Amersham Pharmacia Biotech, Little Chalfont, UK) and total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per kg rice hull.

Total flavonoids content determination The total flavonoid content of samples was determined by Kim *et al.* (13). Briefly, diluted samples (1 mL) and NaNO₂ (0.3 mL, 5%) were mixed and AlCl₃ (10%, 0.3 mL) was added at 5 min and 1 M NaOH (2 mL) was added at 6 min. The absorbance of samples was measured at 510 nm. Total flavonoid content was expressed as milligrams of catechin equivalents (CAE) per kg rice hull.

ABTS radical scavenging activity ABTS radical scavenging activity was determined by previously described method Lee *et al.* (14). The samples (20 µL) were mixed with ABTS radical solution (980 µL) and incubated in a water bath (37°C) for 10 min and the decrease of absorbance at 734 nm was measured.

Cell culture RAW 264.7 mouse macrophage cells (5x10⁵ cells/well) were seeded in 96-well plates and cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with FBS (10%) and penicillin-streptomycin (100 unit/mL) at 37°C in 5% CO₂ for 24 h.

After 24 h, the medium was discarded and the fresh medium containing sample and 5 µg/mL of lipopolysaccharide (LPS) were added and the cells were incubated for additional 24 h. Cell supernatant was collected to determine nitric oxide (NO) content. The 3T3-L1 preadipocytes (8x10⁴ cells/well) were seeded and cultured in DMEM supplemented with bovine calf serum (10%) and penicillin-streptomycin (100 units/mL) at 37°C in 5% CO₂. When the cells were confluent (0 day), they were placed in differentiation medium containing DMEM, fetal bovine serum (FBS, 10%), IBMX (0.5 mM), DEX (1 µM) and insulin (1 µg/mL). After 2 days, the medium was replaced with fresh FBS/DMEM medium containing insulin (1 µg/mL) and sample in 1% DMSO. The medium was changed every 2 days. The cells were harvested after 8 days to determine lipid accumulation.

Cell viability assay Cell viability was evaluated using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. The RAW 264.7 macrophage cells and 3T3-L1 adipocytes cells were cultured for 1 day and 8 days in the presence of sample, respectively. After incubation for 3 h with MTT dye (100 µL, 0.8 mg/mL), dye was removed and formazan crystals were solubilized in DMSO. The absorbance was measured at 540 nm using ELISA (Biotek Instrument Inc., Winooski, VT, USA).

Nitric oxide (NO) production in RAW 264.7 macrophages NO production in the culture media was quantified by the method of Marocchi *et al.* (15). Briefly, culture supernatant (100 µL) was mixed with equal volume of freshly prepared Griess reagent and absorbance was measured at 546 nm using ELISA. The NO was calculated from the standard curve of sodium nitrite prepared in DMEM.

Lipid accumulation in 3T3-L1 adipocytes The lipid accumulation in the mature adipocytes was determined using Oil Red O staining on the experiment day 8. The cells were fixed with 10% formalin at room temperature after washing with phosphate buffered saline (PBS). The fixed cells were stained with 0.21% filtered Oil-Red O solution for 10 min and were rinsed several times with water. The stained lipid droplets were observed with an Olympus microscope (Tokyo, Japan) and representative images were captured. Finally, Oil Red O stained material was eluted with isopropanol (1 mL) and absorbance was measured at 500 nm. The result was expressed as percentage of Oil red O stained material relative to control.

Statistical analysis All assays were conducted in triplicate and the results were expressed as mean values±standard deviations. Statistical analysis was performed by analysis of variance (ANOVA) using SPSS18.0 statistical software (SPSS Inc., Chicago, IL, USA). One-way ANOVA was carried out to determine the treatment effect. Results with significant differences at *p*<0.05 as analyzed by ANOVA were further compared with Duncan's multiple comparison test.

Table 1. Effect of enzymatic hydrolysis and HPP treatment on the extraction yield of triclin, total phenolics and total flavonoids

Sample	Tricin content (mg/kg rice hull)	Total phenolics (mg GAE ¹⁾ / kg rice hull)	Total flavonoids (mg CAE/ kg rice hull)
Control extract ²⁾	14.74±3.53 ^{c3)}	1,783±5.67 ^c	76.16±0.64 ^e
HPP-100 MPa	16.66±2.21 ^c	1,709±17.9 ^c	74.40±11.5 ^e
HPP-250 MPa	16.78±3.07 ^c	1,735±11.6 ^c	71.82±6.14 ^e
HPP -500 MPa ⁴⁾	19.76±2.75 ^{bc}	1,809±11.2 ^c	131.2±4.54 ^d
Celluclast+HPP	32.96±3.65 ^a	3,299±5.66 ^a	183.6±6.15 ^c
Pectinex+HPP	18.85±1.79 ^{bc}	3,364±6.13 ^a	245.6±8.12 ^a
Rapidase+HPP	22.20±0.03 ^b	3,550±3.51 ^a	197.7±7.01 ^b
Viscozyme+HPP	16.51±1.97 ^c	2,861±15.9 ^b	194.3±2.05 ^b

¹⁾GAE: gallic acid equivalent, CAE: catechin equivalent, HPP: high hydrolytic pressure.

²⁾Control extract was prepared by ethanol extraction at 25°C for 24 h under agitation.

³⁾Different letters in the same column indicate significant difference at $p < 0.05$.

⁴⁾HPP treatment was carried out at 500 MPa for 6 min after enzymatic hydrolysis (0.5%, w/w) for 3 h in a 40°C water bath.

Results and Discussion

Effect of combination of enzymatic hydrolysis and HPP treatment on extraction yield of triclin, total phenolics and total flavonoids from rice hull The effects of HPP treatment alone and combination of enzymatic hydrolysis and HPP treatment on extraction yields of triclin and phenolic compounds are shown in Table 1. Enzymatic hydrolysis prior to HPP treatment significantly increased the extraction yield of triclin, total phenolics and total flavonoids compared to that obtained with HPP alone, performed at an elevated hydrostatic pressure of 500 MPa.

The yield of triclin from rice hull varied depending on the type of enzyme used for hydrolysis. Although the effectiveness of enzymes on the release of bioactive compounds depends on the composition and structural complexity of the extraction source, carbohydrate-hydrolyzing enzymes such as pectinase, cellulase, and hemicellulase were reported to improve the extraction of polyphenols (16). For triclin extraction, Celluclast resulted in the highest yield compared to other enzymes. Gottschalk *et al.* (17) reported that cellulase contains endoglucanase (E.C. 3.2.1.4) which breaks down low crystallinity region of cellulose fibers. Cellulase also contain cellobiohydrolase (EC 3.2.1.91) and β -glucosidase (EC 3.2.1.21) activity. However, the combined use of a different enzyme such as Viscozyme along with Celluclast did not enhance triclin extraction (data not shown).

Effect of Celluclast concentration on the extraction yield of triclin obtained by combination of enzymatic hydrolysis and HPP treatment Flavonoids are secondary plant metabolites and are mostly bound to cell wall components such as cellulose, hemicellulose and pectin (18). Outer layers of grass, cereal crops and aromatic region of lignin fraction of agricultural wastes such as rice straw and hull were reported to contain triclin (19). The effect of Celluclast

Fig. 1. The effect of Celluclast concentration on triclin extraction yield from rice hull obtained by combination of enzymatic hydrolysis and HPP treatment. Control extract was obtained by ethanol extraction at 25°C for 24 h under agitation. HPP treatment was carried out at 500 MPa for 6 min after enzymatic hydrolysis using Celluclast at indicated concentrations. Bars with different letters indicate significant difference at $p < 0.05$.

concentration on the extraction yield of triclin was investigated. As shown in Fig. 1, 0.5% Celluclast assisted hydrolysis prior to HPP treatment of rice hull increased the yield of triclin more than 2 folds. This result indicates that combination treatment of enzymatic hydrolysis and HPP was effective for the extraction of bioactive compounds from rigid cell wall matrix.

Effect of rice hull extracts prepared by different extraction methods on ABTS radical scavenging activity

Butsat and Siriamornpun (20) reported that total phenolic content in husk fraction of Thai rice was 1.2~2.2 mg GAE/g sample depending on collection sites. This content was comparable to total phenolic content (1.74 mg GAE/g) extracted from rice hull by conventional solvent extraction in this study. Compared to conventional solvent extraction, both HPP and the combination of enzymatic hydrolysis and HPP treatment significantly increased the yield of total phenolics and flavonoids from rice hull (Fig. 2). The increased extraction yield of total phenolics and flavonoids by HPP treatment was previously reported. The extraction yield of total phenolics from strawberry and blackberry purees significantly increased when extracted at pressure of 500 MPa while heat treatment at 70°C for 2 min did not have a significant effect (21). Xi (22) described the extraction of bioactive compounds from plant resources as a mass transport phenomenon. The extractable solids present in plant matrix migrate into the solvent until they reach equilibrium. The application of pressure reduced average distance between solvated species and accelerated chemical reactions increasing the extraction yield (23). Prasad *et al.* (24) reported no significant difference in the extraction yields of corilagin from longan fruit pericarp as holding pressure time increased from 2.5 to 30 min at 500 MPa. This result indicates that equilibrium of pressure

Fig. 2. The effect of Celluclast concentration on the extraction yield of phenolics and flavonoids from rice hull obtained by combination of enzymatic hydrolysis and HPP treatment. Control extract was obtained by ethanol extraction at 25°C for 24 h under agitation. HPP treatment was carried out at 500 MPa for 6 min after enzymatic hydrolysis using Celluclast at indicated concentrations. Bars with different letters indicate significant difference at $p < 0.05$.

between internal and external sides of the cell is attained in a short time.

In order to examine the effect of extraction methods on antioxidant activity of rice hull extract, ABTS radical scavenging activity of extracts was evaluated. As shown in Fig. 3, the extract prepared by combination of enzymatic hydrolysis and HPP treatment either with 0.25 or 0.5% Celluclast displayed the highest radical scavenging activity. A close relationship between total phenolic content and antioxidant activity was reported. Kim *et al.* (25) examined the antioxidant activity of nine rice hull extracts prepared using 70% ethanol extraction. A strong positive correlation ($R^2=0.97$) between polyphenolic content and ABTS radical scavenging activity was observed whereas no correlation was found between γ -oryzanol content and ABTS radical scavenging activity. Taken together, the increased antioxidant activity was attributable to increased total phenolics and flavonoids content.

Effect of rice hull extracts prepared by different extraction methods on NO production in LPS stimulated RAW 264.7 macrophages

NO and prostaglandin E_2 (PGE₂) mediate inflammatory response. The effect of rice hull extracts on NO production was determined in LPS stimulated RAW 264.7 cells as an indicator of anti-inflammatory activity. NO production was significantly decreased by the extract obtained by combination of 0.5% Celluclast and HPP treatment compared to the extract obtained by control solvent extraction. Extracts at the tested concentration induced no cytotoxic effect (Fig. 4A and 4B). Verschoyle *et al.* (26) reported that tricetin showed better safety profile compared to that of genistein and quercetin and they concluded that tricetin was safe for use in clinical trials as a cancer suppressive agent.

Fig. 3. The effect of rice hull extracts prepared by different extraction methods on ABTS radical scavenging activity. Control extract was obtained by ethanol extraction at 25°C for 24 h under agitation. HPP treatment was carried out at 500 MPa for 6 min after enzymatic hydrolysis using Celluclast at indicated concentrations. Bars with different letters indicate significant difference at $p < 0.05$.

Inducible nitric oxide synthase (iNOS) expressed in the stimulated macrophages and increases NO production during inflammation. NO reacts with superoxide radicals to form highly reactive peroxynitrite. Prolonged excessive NO production was reported to cause tissue damage and detrimental inflammatory disorders (27).

While extract compositions vary depending on extraction condition and procedure, Chung *et al.* (28) identified 7 compounds in rice hull ethyl acetate extract: hentriacontane, 1-tetratriacontanol, β -sitosterol, momilactone A, momilactone B, tricetin, and β -sitosterol-3-O- β -D-glucoside. Nenadis *et al.* (29) examined phenolic composition of rice hull extract obtained using NaOH (4 M) and reported that *p*-coumaric acid and ferulic acid were the major phenolic compounds in the extract. In the present study, tricetin was one of the major flavonoids identified in the extract and small amount of ferulic acid was also identified (data not shown).

In a recent report, anti-inflammatory activities of tricetin and tricetin 4'-O-(threo- β -guaiacylglycerol) ether isolated from Njavara rice were evaluated in LPS stimulated human peripheral blood mononuclear cells and RAW 264.7 macrophages (30,31). The tricetin treatment effectively suppressed the production of NO, PGE₂, TNF- α , and IL-6. The blocking of transcription factor nuclear factor- κ B (NF- κ B) was suggested for major anti-inflammatory mechanism exerted by tricetin.

Effect of rice hull extracts prepared by different extraction methods on lipid accumulation in 3T3-L1 adipocytes

The effect of rice hull extracts prepared by different extraction methods on adipogenesis was evaluated in 3T3-L1 adipocytes and the extent of intercellular lipid accumulation was measured by Oil Red O staining. As shown in Fig. 5A, the intercellular lipid accumulation was significantly decreased by all the extracts. Significant difference was found between extracts prepared by HPP treatment alone and combined treatment of

Fig. 4. The effect of rice hull extracts prepared by different extraction methods on NO production (A) and cell viability (B) in LPS stimulated RAW 264.7 macrophages. NO production and cell viability were determined after 24 h treatment with sample (100 μg/mL) and LPS. Control extract was obtained by ethanol extraction at 25°C for 24 h under agitation. HPP treatment was carried out at 500 MPa for 6 min after enzymatic hydrolysis using Celluclast at indicated concentrations. Bars with different letters indicate significant difference at $p < 0.05$.

enzymatic hydrolysis and HPP compared to control extract. All rice hull extracts displayed no cytotoxic effect on 3T3-L1 cells (Fig. 5B).

Adipogenesis is the transition of fibroblastic preadipocytes to mature adipocytes and is regulated by hormones and transcription factors. It was demonstrated that adipogenesis could be modulated by down regulating the adipogenic transcriptional factors with plant extracts (32). Recently, the authors isolated triclin from oat hull and demonstrated that triclin was a major anti-adipogenic compound in the oat hull extract. The oat hull extract containing triclin effectively suppressed the expression of adipocyte specific transcription factor genes such as peroxisome proliferator-activated receptor γ (PPAR- γ), CCAAT/enhancer binding protein- α (CEBP/ α), and sterol regulatory binding protein 1 (SREBP1) (7). Structurally similar flavonoids such as apigenin (5,7,4'-trihydroxyflavone) and isorhamnetin (5,7,4-trihydroxy-3-methoxyflavone) were reported to exhibit antiadipogenic activity

Fig. 5. The effect of rice hull extracts prepared by different extraction methods on lipid accumulation (A) and cell viability (B) in 3T3-L1 adipocytes. The lipid accumulation and cell viability in the mature adipocytes were determined using Oil Red O staining (100 μg/mL sample) and MTT assay on the experiment day 8. Control extract was obtained by ethanol extraction at 25°C for 24 h under agitation. HPP treatment was carried out at 500 MPa for 6 min after enzymatic hydrolysis using Celluclast indicated concentrations. Bars with different letters indicate significant difference at $p < 0.05$.

(33,34). The mechanism underlying anti-adipogenic effect of triclin at molecular level are currently under investigation.

In conclusion, combination of enzymatic hydrolysis and HPP treatment significantly improved the extraction yield of triclin, total phenolics and flavonoids from rice hull compared to the traditional solvent extraction or HPP treatment alone. The rice hull extract containing triclin showed antioxidant, antiinflammatory, and antiadipogenic activities and the efficacy of rice hull extract prepared by either HPP or combined treatment of enzymatic hydrolysis and HPP was higher than that of the extract prepared by traditional solvent extraction.

Acknowledgments This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (2014R1A2A2A01007169).

Disclosure The authors declare no conflict of interest.

References

- Lim JS, Manan ZA, Alwi SRW, Hashim H. A review on utilisation of biomass from rice industry as a source of renewable energy. *Renew. Sust. Energ. Rev.* 16: 3084-3094 (2012)
- Deng GF, Xu XR, Zhang Y, Li D, Gan RY, Li HB. Phenolic compounds and bioactivities of pigmented rice. *Crit. Rev. Food Sci.* 53: 296-306 (2013)
- Devi RR, Arumughan C. Phytochemical characterization of defatted rice bran and optimization of a process for their extraction and enrichment. *Bioresource Technol.* 98: 3037-3043 (2007)
- Durate-Almeida JM, Novoa AV, Linares AF, Lajolo FM, Genovese MI. Antioxidant activity of phenolics compounds from sugar cane (*Saccharum officinarum* L.) juice. *Plant Food Hum. Nutr.* 61: 187-192(2006)
- Wang HB, Yao H, Bao GH, Zhang HP, Qin GW. Flavone glycosides with immunomodulatory activity from the leaves of *Pleioblastus amarus*. *Phytochemistry* 65: 969-974 (2004)
- Cai H, Hudson EA, Mann P, Verschoye RD, Greaves P, Manson MM, Steward WP, Gescher AJ. Growth inhibitory and cell cycle arresting properties of the rice bran constituent triclin in human-derived breast cancer cells *in vitro* and in nude mice *in vivo*. *Brit. J. Cancer* 91: 1364-1371 (2004)
- Lee D, Park HY, Kim S, Park Y, Bang MH, Imm JY. Antiadipogenic effect of oat hull extract conataing triclin on 3T3-L1 adipocytes. *Process Biochem.* in press (2015)
- Moheb A, Grondin M, Ibrahim RK, Roy R, Sarhan F. Winter wheat hull (husk) is a valuable source for triclin, a potential selective cytotoxic agent. *Food Chem.* 138: 931-937 (2013)
- Mohanlal S, Parvathy R, Shalini V, Helen A, Jayalekshmy A. Isolation, characterization and quantification of triclin and flavonolignans in the medicinal rice Njavara (*Oryza sativa* L.) as compared to staple varieties. *Plant Food Hum. Nutr.* 66: 91-96 (2011)
- Strati IF, Gogou E, Oreopoulou V. Enzyme and high pressure assisted extraction of carotenoids from tomato waste. *Food Bioprod. Process.* 94: 668-674 (2015)
- Xi J, Shen D, Li Y, Zhang R. Miomechanism of ultrahigh pressure extraction of active ingredients from green tea leaves. *Food Control* 22: 1473-1476 (2011)
- Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Method Enzymol.* 299: 152-178 (1999)
- Kim DO, Jeong SW, Lee CY. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chem.* 81: 321-326 (2003)
- Lee JH, Jeon JK, Kim SG, Kim SH, Chun T, Imm JY. Comparative analyses of total phenols, flavonoids, saponins and antioxidant activity in yellow soy beans and mung beans. *Int. J. Food Sci. Tech.* 46: 2513-2519 (2011)
- Marocchi L, Maguire JJ, Droylefaix MT, Packer L. The nitric oxide-scavenging properties of ginkgo biloba extract EGB 761. *Biochem. Bioph. Res. Co.* 201: 748-755 (1994)
- Landbo AK, Meyer AS. Enzyme-assisted extraction of antioxidative phenols from black currant juice press residues (*Ribes nigrum*). *J. Agr. Food Chem.* 49: 3169-3177 (2001)
- Gottschalk LF, Oliveira RA, da Silva Bon EP. Cellulase, xylanases, β -glucosidase and ferulic acid esterase produced by *Trichoderma* and *Aspergillus* act synergistically in the hydrolysis of sugarcane bagasse. *Biochem. Eng. J.* 51: 72-78 (2010)
- Fu YJ, Liu W, Zu YG, Tong MH, Li SM, Yan MM, Effertt T, Luo H. Enzyme assisted extraction of luteolin and apigenin from pigeonpea [*Cajanus cajan* (L.) Millsp.] leaves. *Food Chem.* 111: 508-512 (2008)
- Wu M, Pang J, Lu F, Zhang X, Che L, Xu F, Sun R. Application of new expansion pretreatment method on agricultural waste. Part I: Influence of pretreatment on the properties of lignin. *Ind. Crop. Prod.* 50: 887-895 (2013)
- Butsat S, Siriamornpun S. Antioxidant capacities and phenolic compounds of the husk, bran and endosperm of Thai rice. *Food Chem.* 119: 606-613 (2010)
- Patras A, Brunton NP, Pieve SD, Butler F. Impact of high pressure processing on total antioxidant activity, phenolic, ascorbic acid, anthocyanin content and color of strawberry and blackberry purees. *Innov. Food Sci. Emerg.* 10: 308-313 (2009)
- Xi J. Caffeine extraction from green tea leaves assisted by high pressure processing. *J. Food Eng.* 94: 105-109 (2009)
- Rivalain N, Roquain J, Demazeau G. Development of high hydrostatic pressure in biosciences: Pressure effect on biological structure and potential applications in biotechnologies. *Biotechnol. Adv.* 28: 659-672 (2010)
- Prasad KN, Yang B, Zhao M, Wei X, Jiang Y, Chen F. High pressure extraction of corilagin from longan (*Dimocarpus longan* Lour.) fruit pericarp. *Sep. Purif. Technol.* 70: 41-45 (2009)
- Kim DJ, Oh SK, Chun A, Yoon MR, Hong HC, Choi IS, Lee JS, Yu KW, Kim YK. Evaluation of biological activities of rice husk extracts. *J. Food Sci. Nutr.* 16: 179-183 (2011)
- Verschoye RD, Greaves P, Cai H, Borkhardt A, Broggini, M, D'Incalci M, Riccio E, Doppalapudi R, Kapetanovic IM, Steward WP. Preliminary safety evaluation of the putative cancer chemopreventive agent triclin, a naturally occurring flavone. *Cancer Chemoth. Pharm.* 57: 1-6 (2006)
- Zamora R, Vodovotz Y, Billar TR. Inducible nitric oxide synthase and inflammatory diseases. *Mol. Med.* 6: 347-373 (2000)
- Chung IM, Hahn SJ, Ahmad A. Confirmation of potential herbicidal agents in hulls of rice, *Oryza sativa*. *J. Chem. Ecol.* 31: 1339-1352 (2005)
- Nenadis N, Kyriakoudi A, Tsimidou MZ. Impact of alkaline or acid digestion to antioxidant activity, phenolic content and composition of rice hull extracts. *LWT-Food Sci. Technol.* 54: 207-215 (2013)
- Shalini V, Bhaskar S, Kumar KS, Mohanlal S, Jayalekshmy A, Helen A. Molecular mechanisms of anti-inflammatory action of the flavonoid, triclin from Njavara rice (*Oryza sativa* L.) in human peripheral blood mononuclear cells: Possible role in the inflammatory signaling. *Int. Immunopharmacol.* 14: 32-38 (2012)
- Jung YS, Kim DH, Hwang JY, Yun NY, Lee YH, Han SB, Hwang BY, Lee MS, Jeong HS, Hong JT. Anti-inflammatory effect of triclin 4'-O-(threo- β -guaiacylglyceryl) ether, a novel flavonolignan compound isolated from Njavara on in RAW 264.7 cells and in ear mice edema. *Toxicol. Appl. Pharm.* 277: 67-76 (2014)
- Yun JW. Possible anti-obesity therapeutics from nature-A review. *Phytochemistry* 71: 1625-1641 (2010)
- Ono M, Fujimori K. Antiadipogenic effect of dietary apigenin through activation of AMPK in 3T3-L1 cells. *J. Agr. Food Chem.* 59: 13346-13352 (2011)
- Lee J, Jung E, Lee J, Kim S, Huh S, Kim Y, Kim Y, Byun SY, Kim YS, Park D. Isorhamnetin represses adipogenesis in 3T3L1 Cells. *Obesity* 17: 226-232 (2009)