

ISSN 2076-3921 www.mdpi.com/journal/antioxidants

Supplementary Information

## The Effect of *Lactobacillus plantarum* ATCC 8014 and *Lactobacillus acidophilus* NCFM Fermentation on Antioxidant Properties of Selected *in Vitro* Sprout Culture of *Orthosiphon aristatus* (Java Tea) as a Model Study

Dase Hunaefi <sup>1,2,\*</sup>, Divine N. Akumo <sup>3</sup>, Heidi Riedel <sup>1</sup> and Iryna Smetanska <sup>1,4</sup>

- <sup>1</sup> Department Method in Food Biotechnology, Institute of Food Technology and Food Chemistry, Berlin University of Technology, Königin-Luise Str. 22, 14195 Berlin, Germany; E-Mails: heidiriedel80@yahoo.de (H.R.); smetanska@mailbox.tu-berlin.de (I.S.)
- <sup>2</sup> Department of Food Science and Technology, Bogor Agricultural University, Bogor, Indonesia
- <sup>3</sup> Institute of Biotechnology, Laboratory of Bioprocess Engineering, Berlin University of Technology Ackerstr. 71-76, 13355 Berlin, Germany; E-Mail: akumo2@yahoo.com
- <sup>4</sup> Department of Plant Food Processing, University of Applied Science Weihenstephan-Triesdorf, Steingruber Str. 2, 91746 Weidenbach, Germany
- \* Author to whom correspondence should be addressed; E-Mail: dase.hunaefi@mailbox.tu-berlin.de; Tel.: +49-30-314-712-63; Fax: + +49-30-832-766-3.

| Plant                   | Condition of fermentation    | Results                 |                             |    |                               |  |
|-------------------------|------------------------------|-------------------------|-----------------------------|----|-------------------------------|--|
|                         |                              | Phe                     | FD                          | FL | AA                            |  |
| White cabbage           | L. plantarum CECT 748,       | NA                      | NA                          | NA | Oxygen radical                |  |
| (Brassica oleracea var. | Leuconostoc mesenteroides    |                         |                             |    | absorbance capacity           |  |
| capitata cv. Megaton)   | CECT 219 or a mixed          |                         |                             |    | (ORAC) values                 |  |
| [1].                    | culture of both strains.     |                         |                             |    | (up to 2-fold) and NO         |  |
|                         |                              |                         |                             |    | production inhibitory         |  |
|                         |                              |                         |                             |    | potency (up to 2.6-fold).     |  |
| Oats (Avena sativa L.)  | SSF with A. oryzae var.      | Increased significantly | Increased siginificantly    | NA | Increased siginificantly      |  |
| [2]                     | effuses, A oryzae, and       | (p < 0.05); e.g.        | (p < 0.05); e.g. Oats ethyl |    | (p < 0.05); e.g. Oats         |  |
|                         | A niger on four subfractions | oats water sub fraction | acetate sub fraction from   |    | ethyl acetate sub fraction    |  |
|                         | of oats: n-hexane,           | from 1,580.1 ±62.6 mg   | 3,714.8 ±94.3 mg of rutin   |    | 747.5 $\pm$ 14.6 micromoles   |  |
|                         | ethyl acetate, n-butanol,    | GAE/100 g DW            | equivalents/100 g DW        |    | of Trolox per gram of         |  |
|                         | and water with ethanol as    | (un-fer.) to            | (un-fer.) to                |    | DW (un-fer.) to               |  |
|                         | solvent extractions.         | 3,632.7 ±73.1 mg        | 7,893.1 ±397.3 mg of rutin  |    | $1,687.9 \pm 40.7$ (fer. with |  |
|                         |                              | GAE/100 g DW            | equivalents/100 g DW        |    | A. oryzae).                   |  |
|                         |                              | (fer. with A. oryzae).  | (fer. with A. oryzae).      |    |                               |  |

Tabel S1. Recent investigations on plant fermentation and its effect on antioxidant properties.

Tabel S1. Cont.

| Plant                   | Condition of fermentation                      | Results              |                         |                     |                    |  |  |
|-------------------------|------------------------------------------------|----------------------|-------------------------|---------------------|--------------------|--|--|
|                         |                                                | Phe                  | FD                      | FL                  | AA                 |  |  |
| Soybean [3]             | The steamed soybeans were                      | 253 (0 h) increased  | Increased from          | Total flavonols     | The level of       |  |  |
|                         | let stand for 1 h at 37 °C to                  | to 9,414 mg/kg at    | 53.43mg/kg (0 h) to     | increased           | DPPH radical       |  |  |
|                         | cool down. After, the                          | the end of           | 67.76 mg/kg (12 h)-     | (data divided into  | scavenging         |  |  |
|                         | cooked soybeans was                            | fermentation (60 h). | 73.39 mg/kg (24 h) -    | different type      | activity increased |  |  |
|                         | inoculated with 5% (w/w)                       |                      | 94.32 mg/kg (36 h) -    | flavonols) although | from 53.6 to       |  |  |
|                         | strain Bacillus subtilis CS90                  |                      | 105.30 mg/kg (48 h) –   | flavanol gallates   | 93.9% by 60 h.     |  |  |
|                         | $(1.43 \times 10^7 \text{ cfu/mL})$ and        |                      | 111.98 mg/kg (60 h).    | contents decreased  |                    |  |  |
|                         | fermented for 60 h at 37 °C                    |                      |                         |                     |                    |  |  |
|                         | in incubator and sampled at                    |                      |                         |                     |                    |  |  |
|                         | 0, 12, 24, 36, 48, and 60 h.                   |                      |                         |                     |                    |  |  |
| Anoectochilus           | 5×10 <sup>6</sup> cfu/mL <i>L. acidophilus</i> | Increased; e.g. leaf | NA                      | NA                  | It is clearly      |  |  |
| formosanus Hayata [4]   | BCRC 17002,                                    | (un-fer.) 6.07±1.0   |                         |                     | shown an increase  |  |  |
|                         | Bifidobacterium longum                         | and fermented        |                         |                     | in the detected    |  |  |
|                         | BCRC 14602, L. casei                           | 14.05±1.0 mg/g.      |                         |                     | antioxidant        |  |  |
|                         | subsp. Casei BCRC 12248                        |                      |                         |                     | property may owe   |  |  |
|                         | was inoculated into 100 mL                     |                      |                         |                     | to the increase of |  |  |
|                         | vegetable juice.                               |                      |                         |                     | total phenolic     |  |  |
|                         |                                                |                      |                         |                     | compounds.         |  |  |
| Spirulina               | B.bifidum, L. casei,                           | The results of their | NA                      | NA                  | The greater        |  |  |
| (Arthrospira platensis) | B. infantis, B. longum,                        | study indicated that |                         |                     | quantity of total  |  |  |
| [5]                     | Lactococcus lactis and                         | LAB-fermented        |                         |                     | phenols in         |  |  |
|                         | L.acidophilus.                                 | Spirulina contained  |                         |                     | fermented          |  |  |
|                         |                                                | more polyphenols.    |                         |                     | samples indicates  |  |  |
|                         |                                                |                      |                         |                     | it possesses       |  |  |
|                         |                                                |                      |                         |                     | greater            |  |  |
|                         |                                                |                      |                         |                     | antioxidant        |  |  |
|                         |                                                |                      |                         |                     | activity.          |  |  |
| Graptopetalum           | L. acidophilus BCRC                            | Increased: e.g.      | Increased: e.g. water   | NA                  | The level of       |  |  |
| paraguayense            | 10695, L. plantarum BCRC                       | water extract of     | extract of immature     |                     | antioxidants was   |  |  |
| E. Walther [6]          | 10357 and L. paracasei                         | immature             | G. paraguayense         |                     | significantly      |  |  |
|                         | BCRC 14023.                                    | G. paraguayense      | E. Walther fermentation |                     | increased in       |  |  |
|                         |                                                | E. Walther           | by L. plantarum BCRC    |                     | immature G.        |  |  |
|                         |                                                | fermentation by      | 10357 increased from    |                     | paraguayense       |  |  |
|                         |                                                | L. plantarum         | 17.2 to 22.9 µg/mg.     |                     | E. Walther         |  |  |
|                         |                                                | BCRC 10357           |                         |                     | fermented by       |  |  |
|                         |                                                | increased from 92.2  |                         |                     | L. acidophilus     |  |  |
|                         |                                                | to 111 μg/mg.        |                         |                     | BCRC 10695,        |  |  |
|                         |                                                |                      |                         |                     | L. plantarum       |  |  |
|                         |                                                |                      |                         |                     | BCRC 10357 and     |  |  |
|                         |                                                |                      |                         |                     | L. paracasei       |  |  |
|                         |                                                |                      |                         |                     | BCRC 14023.        |  |  |

Tabel S1. Cont.

|                | Condition of fermentation |                             | Results               |                        |          |                              |  |  |
|----------------|---------------------------|-----------------------------|-----------------------|------------------------|----------|------------------------------|--|--|
| Plant          |                           |                             | Phe                   | FD                     | FL       | AA                           |  |  |
| Codonopsis     | Bifidobacterium longi     | <i>um</i> B6 and <i>L</i> . | The fermentation      | Unlike the total       | NA       | The lowest IC50 values       |  |  |
| lanceolata [7] |                           |                             | process significantly | phenols, fermentation  |          | were 1.25 mg/mL for          |  |  |
|                |                           |                             | increased the total   | decreased the total    |          | high pressure assisted       |  |  |
|                |                           |                             | phenol content of     | flavonoids. The lowest |          | extraction of B. longum      |  |  |
|                |                           |                             | C. lanceolata when    | flavonoid contents     |          | fermented sample and         |  |  |
|                |                           |                             | compared to the       | were observed for high |          | 1.18 mg/mL for               |  |  |
|                |                           |                             | conventional          | pressure assisted      |          | L. rhamnosus fermented       |  |  |
|                |                           |                             | extraction without    | extraction of the      |          | sample, indicating that      |  |  |
|                |                           |                             | fermentation.         | fermented              |          | the fermented                |  |  |
|                |                           |                             | The total phenol      | C. lanceolata with     |          | C. lanceolata extract had    |  |  |
|                |                           |                             | content of            | B. longum              |          | the highest antioxidant      |  |  |
|                |                           |                             | C. lanceolata was the | (0.44 mg RE/g) and     |          | properties.                  |  |  |
|                |                           |                             | highest for high      | L. rhamnosus           |          |                              |  |  |
|                |                           |                             | pressure assisted     | (0.45 mg RE/g). The    |          |                              |  |  |
|                |                           |                             | extraction from       | high pressure assisted |          |                              |  |  |
|                |                           |                             | L. rhamnosus          | extraction of          |          |                              |  |  |
|                |                           |                             | fermented             | un-fermented samples   |          |                              |  |  |
|                |                           |                             | (8.45 mg GAE/g),      | showed a maximum       |          |                              |  |  |
|                |                           |                             | followed by           | flavonoid content of   |          |                              |  |  |
|                |                           |                             | B. longum fermented   | 1.30 mg RE/g,          |          |                              |  |  |
|                |                           |                             | samples               | followed by            |          |                              |  |  |
|                |                           |                             | (8.25 mg GAE/g),      | conventional           |          |                              |  |  |
|                |                           |                             | non-fermented         | extraction of          |          |                              |  |  |
|                |                           |                             | (7.38 mg GAE/g),      | un-fermented sample    |          |                              |  |  |
|                |                           |                             | and conventional      | (0.78 mg RE/g).        |          |                              |  |  |
|                |                           |                             | extraction without    |                        |          |                              |  |  |
|                |                           |                             | fermentation          |                        |          |                              |  |  |
|                |                           |                             | (6.69 mg GAE/g).      |                        |          |                              |  |  |
| Peanuts [8]    | Bifidobacterium           | Gallic acid, caffeic        |                       |                        | Regardl  | ess of the starter organisms |  |  |
|                | longum B17,               | acid, chlorogenic           |                       |                        | used, la | ctic acid fermentation       |  |  |
|                | Lactobacillus casei       | acid and <i>p</i> -coumaric |                       |                        | could hi | ghly improve the DPPH        |  |  |
|                | LC35 and                  | lactic acid                 |                       |                        | radical- | scavenging activity of PF    |  |  |
|                | Lactobacillus             | fermentation resulted       |                       |                        | (Peanut  | Flour). For example, at      |  |  |
|                | acidophilus LA51          | in a decrease in the        |                       |                        | 2 mg/m   | L, the extract from FPF      |  |  |
|                |                           | content of these four       |                       |                        | (Fermer  | nted Peanut Flour) showed    |  |  |
|                |                           | phenolic acids.             |                       |                        | 54.1 to  | 85.6% scavenging activity    |  |  |
|                |                           |                             |                       |                        | on DPP   | H radicals.                  |  |  |

Phe = total phenolics; FD = total flavonoids; FL = total flavonols; and AA: antioxidant activity; SSF = solid state fermentations; and LSF = liquid state fermentations.

## References

1. Martinez-Villaluenga, C.; Peñas, E.; Sidro, B.; Ullate, M.; Frias, J.; Vidal-Valverde, C. White cabbage fermentation improves ascorbigen content, antioxidant and nitric oxide production inhibitory activity in LPS-induced macrophages. *LWT-Food Sci. Technol.* **2012**, *46*, 77–83.

- Cai, S.; Wang, O.; Wu, W.; Zhu, S.; Zhou, F.; Ji, B.; Gao, F.; Zhang, D.; Liu, J.; Cheng, Q. Comparative study of the effects of solid-state fermentation with three filamentous fungi on the Total Phenolics Content (TPC), flavonoids, and antioxidant activities of subfractions from Oats (*Avena sativa* L.). J. Agr. Food Chem. 2011, 60, 507–513.
- 3. Cho, K.M.; Lee, J.H.; Yun, H.D.; Ahn, B.Y.; Kim, H.; Seo, W.T. Changes of phytochemical constituents (isoflavones, flavanols, and phenolic acids) during cheonggukjang soybeans fermentation using potential probiotics *Bacillus subtilis* CS90. *J. Food Compos. Anal.* **2011**, *24*, 402–410.
- 4. Ng, C.-C.; Wang, C.-Y.; Wang, Y.-P.; Tzeng, W.-S.; Shyu, Y.-T. Lactic acid bacterial fermentation on the production of functional antioxidant herbal *Anoectochilus formosanus* Hayata. *J. Biosci. Bioeng.* **2011**, *111*, 289–293.
- 5. Liu, J.-G.; Hou, C.-W.; Lee, S.-Y.; Chuang, Y.; Lin, C.-C. Antioxidant effects and UVB protective activity of Spirulina (*Arthrospira platensis*) products fermented with lactic acid bacteria. *Process Biochem.* **2011**, *46*, 1405–1410.
- 6. Wu, S.-C.; Su, Y.-S.; Cheng, H.-Y. Antioxidant properties of *Lactobacillus*-fermented and non-fermented *Graptopetalum paraguayense* E. Walther at different stages of maturity. *Food Chem.* **2011**, *129*, 804–809.
- He, X.; Zou, Y.; Yoon, W.-B.; Park, S.-J.; Park, D.-S.; Ahn, J. Effects of probiotic fermentation on the enhancement of biological and pharmacological activities of *Codonopsis lanceolata* extracted by high pressure treatment. *J. Biosci. Bioeng.* 2011, *112*, 188–193.
- 8. Wang, N.-F.; Yan, Z.; Li, C.-Y.; Jiang, N.; Liu, H.-J. Antioxidant activity of peanut flour fermented with Lactic Acid Bacteria. *J. Food Biochem.* **2011**, *35*, 1514–1521.

© 2012 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (http://creativecommons.org/licenses/by/3.0/).