Food Research 7 (4) : 204 - 210 (August 2023)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

Antioxidant profile of red oncom, an Indonesian traditional fermented soyfood

*Surya, R. and Romulo, A.

Food Technology Department, Faculty of Engineering, Bina Nusantara University, Jakarta 11480, Indonesia

Article history:
Received: 4 December 2021
Received in revised form: 5 January 2022
Accepted: 21 April 2022
Available Online: 22 August 2023

Keywords:
Oncom,
Tempeh,
Soybean,
Isoflavone,
Antioxidant

DOI:
https://doi.org/10.26656/fr.2017.7(4).650

Abstract

Red oncom is an Indonesian local soyfood traditionally made from by-products of tofu industries through a mould fermentation involving *Neurospora* sp. Red oncom contains isoflavones and carotenoids, bioactive compounds exerting antioxidant activities. This study aimed to investigate the antioxidant activities of red oncom produced through traditional methods compared to unfermented soybeans and tempeh (or tempe), another Indonesian traditional fermented soyfood. Following red oncom extraction using ethanol 95%, several experiments were conducted, including analysis of isoflavones, carotenoids, total phenolics, total flavonoids and free radical scavenging activity. Antioxidant activity appeared to be significantly higher in red oncom than in unfermented soybeans, but lower in comparison to tempeh ($p<0.05$). A longer fermentation period was associated with increased antioxidant activities in red oncom along with decay-related organoleptic issues appearing at day-4. Interestingly, antioxidant activities were found to be stronger on the mouldy surface of red oncom compared to its inner side, mainly due to the presence of moulds biosynthesizing carotenoids and hydrolyzing isoflavone glucosides into aglycones. Thus, it was suggested that the potential antioxidative properties of red oncom could support its development as a functional food.

1. Introduction

Oncom is an Indonesian traditional soyfood whose production involves fermentation by moulds. It is native to West Java and has been involved in Sundanese cuisine for centuries. Oncom is usually made from by-products obtained from the production of other foods, thus increasing the economic efficiency of food production. There are two types of oncom commonly found at traditional markets in Indonesia, mainly in West Java: red oncom and black oncom, both are named so due to their appearance determined by the spore colour of the main moulds used in their fermentation. Red oncom is generally made from soy pulp left from making tofu (also known as okara) through a fermentation process involving *Neurospora* sp. (commonly *N. sitophila*, *N. crassa* and/or *N. intermedia*) while black oncom is made from peanut press cake left after making peanut oil fermented by *Rhizopus oligosporus* and *Mucor* sp. (Surono, 2016). In some cases, red oncom can also be made from peanut press cake fermented using red/orange spore-forming *Neurospora* sp. (e.g., oncom Bandung from West Java). *Rhizopus oligosporus* is also the main microorganism involved in the fermentation of tempeh, an Indonesian traditional soyfood popular among vegetarians worldwide (Ahnan-Winarno *et al.*, 2021; Romulo and Surya, 2021). In this study, the term "oncom" henceforth refers to the common red oncom made from tofu by-products.

Despite being produced from by-products, oncom still possesses interesting nutritional benefits for humans. A hundred grams of oncom dry matter provides about 27 g protein (Matsuo, 1997), which is considered as high since it represents about one-third of daily protein needs for an adult. The proteins in oncom are present in shorter chains called peptides due to mould proteolytic activities, thus increasing protein bioavailability in the human gut. The presence of such peptides is also responsible for the umami taste of oncom (Andayani *et al.*, 2020). *Neurospora* sp. secretes enzymes such as amylases and lipases that digest complex carbohydrates and fats, resulting in the formation of alcohol and esters contributing to the distinguished flavours of oncom (Kuligowski *et al.*, 2017). Enzyme a-galactosidase breaks down the flatulence-causing oligosaccharides in soybeans, thus making oncom more gut-friendly (Worthington and Beuchat, 1974). *Neurospora* sp. is also

*Corresponding author.
Email: *reggie.surya@binus.edu*

eISSN: 2550-2166
Published by Rynnye Lyan Resources

able to degrade aflatoxin, a carcinogenic mycotoxin found mainly in some agricultural crops such as cereals and grains (Nout, 1989).

Oncom is believed to exert antioxidant activities due to the presence of isoflavones and carotenoids. Isoflavones are phytochemicals found mainly in leguminous that are able to act as antioxidants and reduce oxidative stress. Isoflavones have been linked to the prevention of several diseases, including hypercholesterolemia, atherosclerosis, cardiovascular disease, cancer, osteoporosis and relief of menopausal symptoms in women (Zaheer and Akhtar, 2017). Carotenoids, the red/orange pigment expressed by *Neurospora* sp., is a group of potent liposoluble antioxidants possessing a long polyene chain with conjugated double bonds allowing electron transfer throughout the molecule. The most abundant carotenoids constituting the colour of *Neurospora* sp. are neurosporaxanthin, lycopene and g-carotene (Hornero-Méndez *et al.*, 2018)

This study aimed to analyze the isoflavone profile and antioxidant activities of oncom in comparison with cooked soybeans and tempeh. The influence of oncom fermentation time (2-4 days) on antioxidant profile and activities was also determined. Furthermore, since carotenoids are present mostly on the surface of oncom where the moulds grow, antioxidant profile and activities were also analyzed separately between the surface (mouldy side) and the inner side of oncom.

2. Materials and methods

2.1 Sample preparation

Soybeans (var Anjasmoro, harvest age 85 days, produced by UD Sumber Makmur, Nganjuk, East Java, Indonesia) were used as the main materials for producing tempeh and oncom. As standard, soybeans were cleaned, cooked by boiling for 30 mins and husked manually. Tempeh was produced according to Surya *et al.* (2021) using a starter containing a mixture of *R. oligosporus* and *R. oryzae* (Brand Unggul, produced by PD Sukma Jaya, Tegal, Central Java, Indonesia, 5 g/kg soybean). The inoculated soybeans underwent a fermentation process for 36 h at room temperature (25^{o}C) to produce raw tempeh. Oncom fermentation was done according to the procedures provided by a local producer. Briefly, soybeans were cleaned, boiled for 30 min, husked manually and extracted with hot water by using a blender (ratio water:soybeans = 8:1 (v/w)). The soy pulp was then separated from the extract using a cheesecloth and squeezed to remove the maximum amount of water. Afterwards, tapioca starch (Brand Rose Brand, 20 g/kg soy pulp) and commercial vinegar (Brand Dixi, 5 mL/kg soy pulp) were added to the soy pulp, mixed thoroughly and the mixture was steamed for 1 hr. After cooling down, the mixture formed a more solid mass called cake. The cake was inoculated with oncom starter containing spores of *N. sitophila*, *N. crassa* and *N. intermedia* (ratio 1:1:1, 20 g/kg cake), wrapped in banana leaves and left fermented at room temperature (25^{o}C) for 48, 72 and 96 hrs.

To produce oncom extracts, raw oncom was cut into smaller pieces and mixed with distilled water or ethanol 95% with a ratio of 1:3 (w/v) by using a blender, resulting in a puree. The puree was then filtered by using a cheesecloth to separate the pulp from the filtrate. The filtrate was collected and kept in a freezer (-20^{o}C) for further analysis. Soybean and tempeh extracts were prepared using the same method as oncom extracts. To analyze the difference between the mouldy surface side and the inner side of oncom, the mouldy sides of oncom fermented for 72 hrs were sliced using a knife with a thickness of approximately 1.5 cm. Afterwards, each separate side of oncom was extracted using the same protocol mentioned above.

2.2 Chemical composition analysis

2.2.1 Proximate analysis

Proximate analysis was performed using methods established by the Association of Official Analytical Chemists (AOAC) and American Oil Chemists Society (AOCS) according to Maisarah *et al.* (2014). The moisture was measured by gravimetry following oven-drying at 135^{o}C for 2 hrs. The ash was determined by calcination at 550^{o}C. The protein level was analyzed by the Kjehldahl method. Lipids were extracted in the Soxhlet apparatus with petroleum ether at 40-60^{o}C. Carbohydrates were determined by difference.

2.2.2 Analysis of isoflavones and carotenoids

Both analyses were performed by using FlowCal 5000 HPLC Liquid Flow Meter (Tovatech, Maplewood, New Jersey) with a C-18 column (Isogen Life Science, Utrecht, Netherlands). The injected sample size was 20 mL with a flow rate of 1 mL/min at 35^{o}C. For analysis of isoflavones (Sun *et al.*, 2011), a mobile phase consisting of acetonitrile (13-30%) and 0.1% acetic acid was applied with linear gradient elution. For analysis of carotenoids (Jin *et al.*, 2010), the sample was eluted by chloroform: methanol 3:1 (v/v) as a mobile phase. The components were determined using Biochrom Libra S60 Double Beam UV-Vis Spectrophotometer (Biochrom, Cambridge, UK) at 255 nm (for isoflavones) and 450 nm (for carotenoids). The concentration of each component in the samples was deduced by comparing the peak area of each component with the predefined concentrations of standard solutions.

https://doi.org/10.26656/fr.2017.7(4).650

2.2.3 Total phenolics, total flavonoids and free radical scavenging activity

All assays were performed by spectrophotometric methods as previously described (Surya and Romulo, 2020). Total phenolics and total flavonoids were done by using reagent Folin-Ciocalteau and aluminium chloride respectively. The results were expressed as gallic acid equivalents (GAE) for total phenolics and quercetin equivalents (QE) for total flavonoids. Free radical scavenging activities were analyzed using a,a-diphenyl-b -pricryl-hydrazyl (DPPH) radicals. Antioxidant activities were expressed as a percentage of free radical inhibition obtained by using the following formula : (Acont-Atest)/ Acont]×100%, where Acont is the absorbance of DPPH solution and Atest is the absorbance of DPPH and sample mixture solution.

2.3 Statistical analysis

Data ($n \geq 3$) were analyzed by using the software GraphPad Prism 4 for Windows. All data were reported as mean±SD. The influence of different samples extracted using the same solvent was analyzed by one-way ANOVA followed by Dunnett's test in case of significant differences ($p<0.05$). Analysis of the difference between water extract and ethanol extract was done by student's t-test ($p<0.05$).

3. Results and discussion

Table 1 features the proximate composition of soybeans, tempeh and oncom. Water is present in oncom at relatively high concentrations (84-89%), thus rendering it extremely perishable and prone to microbial decay. Oncom contains approximately 4-6% protein, 2-3% fat and 3-4% carbohydrate. Based on the calculation using a dry basis, oncom is as nutritious as soybeans and tempeh, with about 40% of its dry matter consisting of protein. During oncom fermentation, no significant nutrient loss was observed, as shown by the relatively constant concentration of dry matter (ash, protein, fat and carbohydrate) of oncom compared to soybeans. However, the nutrients undergo changes during oncom fermentation, particularly degradation and hydrolysis due to mould activities.

Antioxidant activity is an essential parameter of food functionality since oxidative stress and free radicals are widely correlated with health disorders including cardiovascular disease, atherosclerosis, neurodegenerative diseases, diabetes mellitus, cancer and ageing-related functional declines (Zhang *et al.*, 2015). This study analyzed the antioxidant activities of soybeans, tempeh and oncom extracted in distilled water or ethanol. The choice of solvents used was based on Chang *et al.* (2009) who previously reported that tempeh extract in water and ethanol exhibited greater antioxidant activities compared to tempeh extract in nonpolar solvents such as hexane, petroleum ether or ether.

Figure 1A-C shows higher total phenolics, total flavonoids and free radical scavenging activities in ethanol extracts compared to water extracts. According to Bustamante-Rangel *et al.* (2018), isoflavones have greater solubility in slightly less polar solvents such as methanol or ethanol than in water. Therefore, there could have been a higher concentration of isoflavones extracted in the ethanol compared to water contributing to such findings. In general, total phenolics, total flavonoids and free radical scavenging activities of oncom, regardless of fermentation time, were higher than soybeans but lower than tempeh. According to Figure 1C, oncom appeared to neutralize 10.2-16.7% of 1 mmol DPPH radical activities while soybeans and tempeh scavenge 7.5% and 25.6% of DPPH activities respectively. However, such antioxidant activities of

Table 1. Proximate analysis of ethanol extracts of soybeans, oncom and tempeh.

	Soybean (cooked)	Oncom 48 hrs	Oncom 72 hrs	Oncom 96 hrs	Tempeh
Wet basis					
Water (% wb)	54.27^{a}	84.21^{c}	85.36^{c}	88.63^{d}	63.23^{b}
Ash (% wb)	0.38^{d}	0.18^{b}	0.16^{b}	0.13^{a}	0.32^{c}
Protein (% wb)	19.70^{d}	6.24^{b}	5.86^{b}	4.48^{a}	17.79^{c}
Fat (% wb)	12.03^{d}	4.34^{b}	4.11^{b}	2.88^{a}	8.85^{c}
Carbohydrate (by difference, % wb)	13.62^{d}	5.03^{b}	4.51^{b}	3.88^{a}	9.81^{c}
Dry basis					
Water (% db)	118.67^{a}	533.31^{c}	583.06^{c}	779.51^{d}	171.96^{b}
Ash (% db)	0.83^{a}	1.14^{b}	1.09^{b}	1.14^{b}	0.87^{a}
Protein (% db)	43.08^{b}	39.52^{a}	40.03^{ab}	39.40^{a}	48.38^{c}
Fat (% db)	26.31^{a}	27.48^{ab}	28.07^{b}	25.33^{a}	24.07^{a}
Carbohydrate (by difference, % db)	29.78^{b}	31.86^{bc}	30.81^{b}	34.12^{c}	26.68^{a}

Values are presented as mean±SD of triplicates (n = 3). Values with different superscripts within the same row are statistically significantly different ($p<0.05$).

https://doi.org/10.26656/fr.2017.7(4).650

oncom and tempeh were considered to be relatively low when compared to vitamin C, a well-known potent antioxidant. Our data showed that as little as 8 mg of vitamin C was able to neutralize 100% of 1 mmol DPPH radical activities.

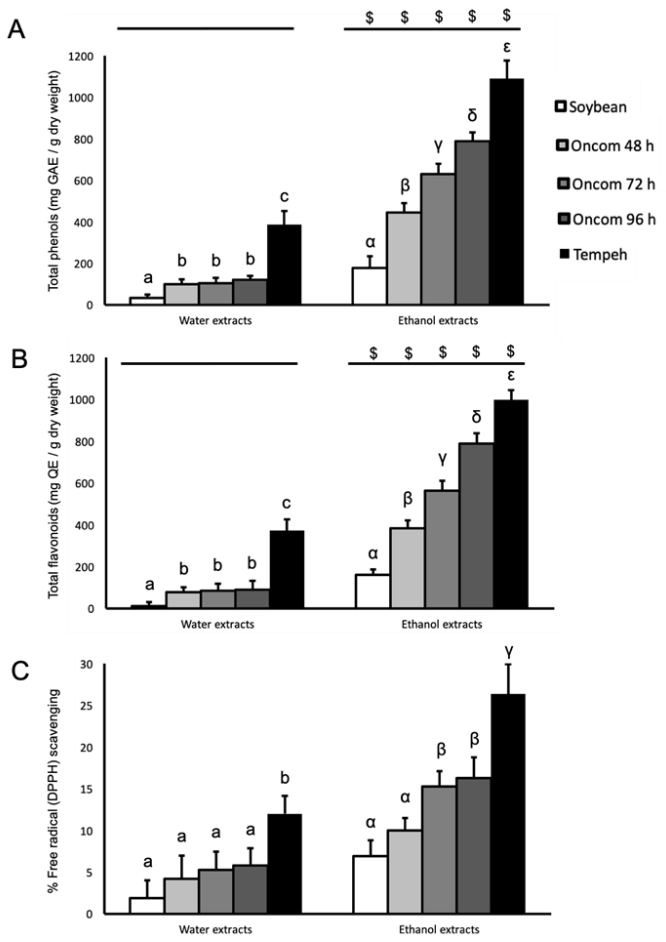

Figure 1. Total phenolics (A), total flavonoids (B) and free radical scavenging activities (C) of water and the ethanol extracts of soybeans, oncom and tempeh. Values are presented as mean±SD of triplicates (n = 3). Bars with different notations within the same group, i.e., water extract or ethanol extract, are statistically significantly different ($p<0.05$). $^{\$}$Statistically significantly different between water extract and ethanol extract ($p<0.05$).

Longer fermentation time was associated with an increase in total phenolics, total flavonoids and free radical inhibitory activities in oncom, notably in ethanol extracts (Figure 1A-C). The same phenomenon was previously reported in several studies regarding tempeh fermentation (Watanabe *et al.*, 2007; Chang *et al.*, 2009). In tempeh fermentation, the increase in antioxidant profile and activities was related to the activity of b-glucosidase secreted by *R. oligosporus* that hydrolyzes isoflavone glucosides (IFGs) into sugar molecules and isoflavone aglycones (IFAs) exerting more potent antioxidant activities (Lee *et al.*, 2005). The highest antioxidant activities were observed in oncom fermented for 96 hrs. However, this oncom exhibited some rot-related organoleptic issues with this oncom including tangy odour and a slightly slimy surface. A decline in pH was also observed during the fermentation, from 5.2 (oncom fermented for 48 hrs) to 3.4 (oncom fermented for 96 hrs). Such a decrease in pH was likely to be caused by further microbial degradation processes resulting in the neoformation of organic acids and other volatile compounds. Oncom fermented for 48 hrs and 72 hrs were organoleptically acceptable.

As presented in Table 2, oncom contains approximately 320 mg isoflavones/100 g dry matter (equivalent to approximately 48 mg/100 g on a wet basis). Despite containing lower total isoflavones compared to soybeans (375 mg/100 g db), antioxidant profile and activities in oncom were found to be higher compared to soybeans (Figure 1A-C). Indeed, during oncom fermentation, a shift in the proportion of IFGs and IFAs reported to total isoflavones was observed (Figure 2). As the proportion of IFGs decreased during oncom fermentation, the proportion of IFAs increased, probably due to the activity of mould b-glucosidase. The IFGs/IFAs ratio declined constantly during oncom fermentation, from 2.25 (unfermented soybeans) to 1.54, 1.28 and 0.92 for oncom fermented for 48 hrs, 72 hrs and 96 hrs respectively. The drop in IFGs/IFAs ratio was also observed to a greater extent during tempeh fermentation from 2.25 to 0.85 following a 48 hr-fermentation.

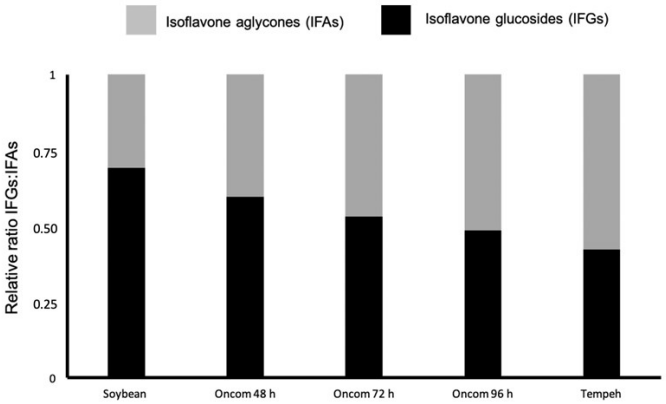

Figure 2. Relative ratio of isoflavone aglycones (IFAs) and isoflavone glucosides (IFGs) of the ethanol extracts of soybeans, oncom and tempeh. Concentrations of IFAs and IFGs are reported to total isoflavones whose value is set to 1. IFGs/IFAs ratios among samples are significantly different ($p<0.05$).

According to Table 3, the mouldy side of oncom exhibited higher total phenolics, total flavonoids and free radical inhibitory activities compared to the inner side of oncom. Such findings proved indirectly that fungal activities were responsible for improving the antioxidant potentials in oncom. Thus, it was suggested that IFAs and carotenoids, present in a greater amount on the surface of oncom, would be the main contributors to the antioxidant activities of oncom.

Oncom exhibits a reddish appearance due to carotenoids, a group of natural pigments present in the

https://doi.org/10.26656/fr.2017.7(4).650

Table 2. Isoflavone profile of ethanol extracts of soybeans, oncom and tempeh.

	Soybean (cooked)	Oncom 48 hrs	Oncom 72 hrs	Oncom 96 hrs	Tempeh
Daidzin (mg/100 g db)	63.24[a]	61.44[a]	63.03[a]	66.85[ab]	67.30[b]
Genistin (mg/100 g db)	196.32[d]	132.56[c]	115.12[b]	87.27[a]	92.83[a]
Total glucosides (mg/100 g db)	259.56[d]	194.00[c]	178.15[b]	154.12[a]	160.13[a]
Daidzein (mg/100 g db)	45.78[a]	41.55[a]	58.35[b]	62.63[b]	74.83[c]
Genistein (mg/100 g db)	69.54[a]	66.62[a]	85.57[b]	105.48[c]	113.54[d]
Total aglycones (mg/100 g db)	115.32[a]	126.17[ab]	138.92[c]	168.11[d]	188.37[e]
Total isoflavones (mg/100 g db)	374.88[c]	320.17[a]	317.07[a]	322.23[a]	348.5[b]
Ratio glucosides:aglycones	2.25[e]	1.54[d]	1.28[c]	0.92[b]	0.85[a]

Values are presented as mean±SD of triplicates (n = 3). Values with different superscripts within the same row are statistically significantly different ($p<0.05$).

Table 3. Antioxidant profile and activities, isoflavone profile and carotenoid profile of ethanol extracts of oncom fermented for 72 hrs.

	Oncom	Moldy side	Inner side
Antioxidant profile and activities			
Free radical scavenging activities (%)	16.12[b]	24.85[c]	6.67[a]
Total phenolics (mg GAE/100 g db)	645.62[b]	1123.08[c]	450.38[a]
Total flavonoids (mg QE/100 g db)	578.43[b]	925.60[c]	377.26[a]
Isoflavone profile			
Daidzin (mg/100 g wb)	68.25[b]	40.17[a]	64.39[b]
Genistin (mg/100 g wb)	123.18[b]	79.82[a]	137.66[c]
Total glucosides (mg/100 g wb)	191.43[b]	119.99[a]	202.05[b]
Daidzein (mg/100 g wb)	52.16[b]	78.36[c]	37.93[a]
Genistein (mg/100 g wb)	84.48[b]	70.45[a]	91.09[c]
Total aglycones (mg/100 g wb)	136.64[a]	148.81[b]	129.02[a]
Total isoflavones (mg/100 g wb)	328.07[b]	268.80[a]	331.07[b]
Ratio glucosides:aglycones	1.40[b]	0.81[a]	1.57[c]
Carotenoid profile			
Beta-carotene (ppm)	85.16[b]	193.23[c]	18.54[a]
Gamma-carotene (ppm)	41.77[b]	123.90[c]	16.69[a]
Lycopene (ppm)	247.58[b]	446.75[c]	28.93[a]
Neurosporaxanthin (ppm)	17.35[b]	43.24[c]	4.18[a]

Values are presented as mean±SD of triplicates (n = 3). Values with different superscripts within the same row are statistically significantly different ($p<0.05$).

spores of *Neurospora* sp. Carotenoids are prominent molecules bestowed with antioxidative properties and have been linked with the prevention of chronic diseases related to oxidative stress (Krinsky and Johnson, 2005). Unlike *R. oligosporus* which unifies integrally soybeans with its mycelium, *Neurospora* sp. is mainly present on the surface of oncom, but not on the inner side. Table 3 demonstrates that isoflavones were more abundant on the inner side of oncom, but IFGs/IFAs ratio was higher on the surface (mouldy side) of oncom due to the enzymatic activities of *Neurospora* sp.

As suspected, due to the presence of the spores of *Neurospora* sp., the levels of carotenoids were higher on the surface of oncom, approximately ten times as high as the levels found in the inner side of oncom (Table 3). Previous findings of Hornero-Méndez *et al.* (2018) revealed neurosporaxanthin, lycopene and g-carotene as the most abundant carotenoids biosynthesized by *N. crassa* while b-carotene was merely present as a minor compound. In this study, contradictorily, lycopene was found to be the carotenoid present at the highest concentration, followed by b-carotene, g-carotene and neurosporaxanthin at the lowest levels (Table 3). Further studies are needed to explain such a contradictory phenomenon. Nevertheless, it is noteworthy that the starter used to produce our oncom was a mixture of *N. sitophila*, *N. crassa* and *N. intermedia* grown on a solid soybean-based medium while the culture used in the experiment of Hornero-Méndez *et al.* (2018) was pure *N. crassa* grown in a liquid medium. The different conditions applied might have contributed to the difference in the composition of fungal carotenoids. Avalos *et al.* (2012) reported that the biosynthesis of neurosporaxanthin in *Neurospora* sp. mainly involves lycopene, but not b- or g-carotene, as an intermediate

https://doi.org/10.26656/fr.2017.7(4).650

molecule. Therefore, in the fungi used to produce our oncom, there could have been a possible shifting in the biosynthesis pathway of neurosporaxanthin for an unknown reason resulting in the accumulation of b- and g-carotene without further conversion to neurosporaxanthin. A deviation of neurosporaxanthin pathway towards b-carotene biosynthesis in fungus *Fusarium fujikuroi* was previously observed by Prado-Cabrero *et al.* (2009). Such a phenomenon ensued due to a point mutation in the gene *CarB* expressing enzymes catalyzing in part the biotransformation of g-carotene to neurosporaxanthin. Further studies need to be conducted in order to investigate whether gene mutations can be responsible for the accumulation of lycopene, b- and g-carotene in our culture.

Taken together, the results obtained in this study give an early insight into the development of oncom as a functional food. The functionality of oncom highlighted in this study was its antioxidant activities that are higher than unfermented soybeans due to the presence of IFAs and carotene in a greater amount. Several studies have been conducted to investigate the potential health benefits of oncom with regard to its antioxidative properties. Matsuo (2002a) reported that IFAs and carotene in oncom fermented using *N. intermedia* suppressed lipid peroxidation in the serum and liver of rats by exerting collaboratively antioxidant activities. In rats fed with a cholesterol-free diet, consumption of oncom led to a reduction in plasma cholesterol (Matsuo, 2002b). Furthermore, oncom was applied as an additional ingredient in miso-like seasonings. *In vivo*, these seasonings have been proven to improve redox state and cholesterolemia in rats (Matsuo, 2004a).

4. Conclusion

Oncom, despite being made from by-products from making tofu, still possesses interesting antioxidative properties due to the presence of carotenoids and isoflavones. It exhibits a higher antioxidant profile and activities compared to unfermented soybeans. Such a phenomenon is associated with mould activities during fermentation since longer fermentation time tended to increase antioxidant activities in oncom.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

The authors would like to thank The Research and Technology Transfer Office (RTTO) of Bina Nusantara University for the help given during the realization of this project.

References

Ahnan-Winarno, A.D., Cordeiro, L., Winarno, F.G., Gibbons, J. and Xiao, H. (2021). Tempeh: A semicentennial review on its health benefits, fermentation, safety, processing, sustainability, and affordability. *Comprehensive Reviews in Food Science and Food Safety*, 20(2), 1717-1767. https://doi.org/10.1111/1541-4337.12710

Andayani, S.N., Lioe, H.N., Wijaya, C.H. and Ogawa, M. (2020). Umami fractions obtained from water-soluble extracts of red oncom and black oncom—Indonesian fermented soybean and peanut products. *Journal of Food Science*, 85(3), 657-665. https://doi.org/10.1111/1750-3841.14942

Avalos, J., Prado-Cabrero, A. and Estrada, A.F. (2012). Neurosporaxanthin production by *Neurospora* and *Fusarium*. In *Microbial Carotenoids from Fungi*, p. 263-274. Totowa, New Jersey, USA : Humana Press. https://doi.org/10.1007/978-1-61779-918-1_18

Bustamante-Rangel, M., Delgado-Zamarreño, M.M., Pérez-Martín, L., Rodríguez-Gonzalo, E. and Domínguez-Álvarez, J. (2018). Analysis of isoflavones in foods. *Comprehensive Reviews in Food Science and Food Safety*, 17(2), 391-411. https://doi.org/10.1111/1541-4337.12325

Chang, C.T., Hsu, C.K., Chou, S.T., Chen, Y.C., Huang, F.S. and Chung, Y.C. (2009). Effect of fermentation time on the antioxidant activities of tempeh prepared from fermented soybean using *Rhizopus oligosporus*. *International Journal of Food Science and Technology*, 44(4), 799-806. https://doi.org/10.1111/j.1365-2621.2009.01907.x

Hornero-Méndez, D., Limón, M.C. and Avalos, J. (2018). HPLC analysis of carotenoids in neurosporaxanthin-producing fungi. In *Microbial Carotenoids*, p. 269-281. New York, USA: Humana Press. https://doi.org/10.1007/978-1-4939-8742-9_16

Jin, J.M., Lee, J. and Lee, Y.W. (2010). Characterization of carotenoid biosynthetic genes in the ascomycete *Gibberella zeae*. *FEMS Microbiology Letters*, 302 (2), 197-202. https://doi.org/10.1111/j.1574-6968.2009.01854.x

Krinsky, N.I. and Johnson, E.J. (2005). Carotenoid actions and their relation to health and disease. *Molecular Aspects of Medicine*, 26(6), 459-516. https://doi.org/10.1016/j.mam.2005.10.001

Kuligowski, M., Pawłowska, K., Jasińska-Kuligowska, I. and Nowak, J. (2017). Isoflavone composition, polyphenols content and antioxidative activity of soybean seeds during tempeh fermentation. *CyTA-Journal of Food*, 15(1), 27-33. https://doi.org/10.1080/19476337.2016.1197316

Lee, C.H., Yang, L., Xu, J.Z., Yeung, S.Y.V., Huang, Y. and Chen, Z.Y. (2005). Relative antioxidant activity of soybean isoflavones and their glycosides. *Food Chemistry*, 90(4), 735-741. https://doi.org/10.1016/j.foodchem.2004.04.034

Maisarah, A.M., Asmah, R. and Fauziah, O. (2014). Proximate analysis, antioxidant and antiproliferative activities of different parts of *Carica papaya*. *Journal of Nutrition and Food Science*, 4(2), 1-7.

Matsuo, M. (1997). Preparation and components of okara -ontjom, a traditional Indonesian fermented food. *Nippon Shokuhin Kogyo Gakkai-Shi*, 44(9), 632-639. https://doi.org/10.3136/nskkk.44.632

Matsuo, M. (2002a). Antioxidant activity of hydrophilic compounds of defatted soybean fermented with *Neurospora intermedia* (D-Ontjom). *Food Science and Technology Research*, 8(3), 235-238. https://doi.org/10.3136/fstr.8.235

Matsuo, M. (2002b). Plasma cholesterol reducing effect by okara oncom (fermented with *Neurospora intermedia*) in rats fed a cholesterol-free diet. *ITE Letters on Batteries, New Technologies and Medicine*, 3(1), 732-735.

Matsuo, M. (2004). Low-salt O-miso produced from koji fermentation of oncom improves redox state and cholesterolemia in rats more than low-salt soybean-miso. *Journal of Nutritional Science and Vitaminology*, 50(5), 362-366. https://doi.org/10.3177/jnsv.50.362

Nout, M.J.R. (1989). Effect of *Rhizopus* and *Neurospora* spp. on growth of *Aspergillus flavus* and *A. parasiticus* and accumulation of aflatoxin B1 in groundnut. *Mycological Research*, 93(4), 518-523. https://doi.org/10.1016/S0953-7562(89)80046-2

Prado-Cabrero, A., Schaub, P., Díaz-Sánchez, V., Estrada, A.F., Al-Babili, S. and Avalos, J. (2009). Deviation of the neurosporaxanthin pathway towards β-carotene biosynthesis in *Fusarium fujikuroi* by a point mutation in the phytoene desaturase gene. *The FEBS Journal*, 276(16), 4582-4597. https://doi.org/10.1111/j.1742-4658.2009.07164.x

Romulo, A. and Surya, R. (2021). Tempe: A traditional fermented food of Indonesia and its health benefits. *International Journal of Gastronomy and Food Science*, 26(1), 100413. https://doi.org/10.1016/j.ijgfs.2021.100413

Sun, J.M., Sun, B.L., Han, F.X., Yan, S.R., Yang, H. and Akio, K. (2011). Rapid HPLC method for determination of 12 isoflavone components in soybean seeds. *Agricultural Sciences in China*, 10 (1), 70-77. https://doi.org/10.1016/S1671-2927(11)60308-8

Surono, I.S. (2016). Ethnic fermented foods and beverages of Indonesia. In Tamang, J.P. (Ed.), Ethnic Fermented Foods and Alcoholic Beverages of Asia, p. 341-382. New Delhi, India: Springer. https://doi.org/10.1007/978-81-322-2800-4_14

Surya, R. and Romulo, A. (2020). Steaming process does not affect the antioxidant activities of tempeh ethanol extract. *Journal of Physics: Conference Series*, 1655 (1), 012023. https://doi.org/10.1088/1742-6596/1655/1/012023

Surya, R., Romulo, A. and Suryani, Y. (2021). Tempeh extract reduces cellular ROS levels and upregulates the expression of antioxidant enzymes. *Food Research*, 5(3), 121-128. https://doi.org/10.26656/fr.2017.5(3).560

Watanabe, N., Fujimoto, K. and Aoki, H. (2007). Antioxidant activities of the water-soluble fraction in tempeh-like fermented soybean (GABA-tempeh). *International Journal of Food Sciences and Nutrition*, 58(8), 577-587. https://doi.org/10.1080/09637480701343846

Worthington, R.E. and Beuchat, L.R. (1974). Alpha-galactosidase activity of fungi on intestinal gas-forming peanut oligosaccharides. *Journal of Agricultural and Food Chemistry*, 22(6), 1063-1066. https://doi.org/10.1021/jf60196a011

Zaheer, K. and Akhtar, M.H. (2017). An updated review of dietary isoflavones: Nutrition, processing, bioavailability and impacts on human health. *Critical Reviews in Food Science and Nutrition*, 57(6), 1280-1293. https://doi.org/10.1080/10408398.2014.989958

Zhang, Y.J., Gan, R.Y., Li, S., Zhou, Y., Li, A.N., Xu, D.P. and Li, H.B. (2015). Antioxidant phytochemicals for the prevention and treatment of chronic diseases. *Molecules*, 20(12), 21138-21156. https://doi.org/10.3390/molecules201219753

