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Okara–by-product from soy processing: characteristic, properties, benefits, and potential perspectives for industry

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Abstract: A by-product from processing of soy into drinks and tofu is the insoluble portion of soybeans, a high-fiber product called okara. With the growing interest in plant substitutes for meat and milk, which are produced mainly from soy, the amount of this by-product, which is often considered waste, is also increasing. Its processing then causes considerable financial and environmental problems. In addition to fiber, okara is rich in proteins, fats, micronutrients, and various phytochemicals. However, these are often in an unavailable form and, in addition, due to okara's high water content, it is easily perishable. Therefore, this review article aimed to gather information on the nutritional composition of the okara, possible adjustments to make unavailable nutrients available, and stabilization at the end of its new incorporation into the food chain either in the capacity of soil amendments and fertilizer to improve food quality and size or directly as a food ingredient.

Introduction

Global soybean production was 363.4 thousand tonnes in the 2019/2020 season. Its production is recovering from a significant decline last season because of limitations imposed by the COVID-19 outbreak and is slightly below the historical high recorded in 2018/2019. In the 2020/2021 season, production in the northern hemisphere is expected to increase in the main producer countries. In the USA, it is reported at the level of 112.5 million tons, which is associated with the desired weather and recovery of planting. In China, soybean production has been growing for seven consecutive seasons, mainly due to government support. Production is also recovering in India thanks to higher and better yields. In contrast, mixed results are observed in the southern hemisphere. In Argentina due to the lower planting and dry wheather crops decline, by in Brasil the crops are very big due to intensive planting and higher yields (FAO, 2021). In the European Union, the food industry and agro-sector produce 370 million tons of waste yearly. Germany, the United Kingdom, Italy, France, and Spain are the largest waste producers are (Correddu et al., 2020). Wastewater from food sector is also environmental problem due to the carbohydrate, carbon source, fats, phosphorus substances and many nitrogenous substances content (Gupta and Pawar, 2018). The certain wastes and by-products from the food industry can contain a higher concentration of bioactive chemicals (Wadhwa et al., 2015; Faustino et al., 2019).

The soy bean is perfect food sources of proteins and for producing Asian foods – dishes and desserts are one of the main ingredients (Rehman et al., 2017). Converse-

Corresponding Author: eva.ivanisova@uniag.sk ly, this is not the case in making some dishes, especially during the soy milk isolation. As a result, a large amount of a by-product called okara is produced. The processing portion, approximately 45% of the product and 55% of okara are produced from a kilo of soybeans (Li et al., 2012). The soybean processing is shown in Figure 1. Although okara is still a nutritionally rich by-product, due to its properties such as low shelf life, rough texture, and bland taste, it is disposed of in landfills or incinerators (Rehman et al., 2017). The high water content makes the disposal procedure more difficult as well as amount of CO_2 generated during combustion. Unprocessed okara very easy perishables and can be a source of odour.

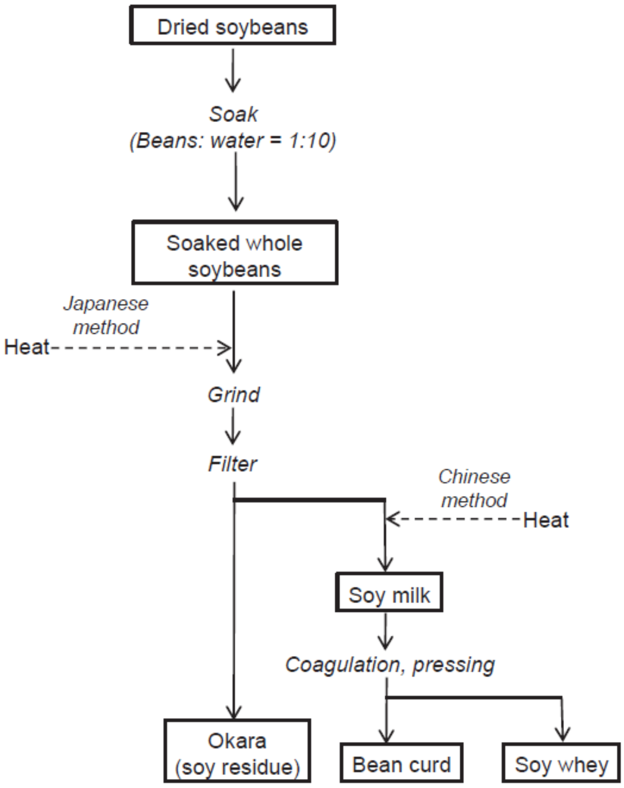

Figure 1. Production of main and by-products of soybeans (Vong and Liu, 2016a)

Soy

There is a many beneficial effects to human health regarding to soy and soy-related products consumption. (Redondo-Cuenca et al., 2007). Soybean (Glycine max L.) is one of the main industrial and globally used food crops. Thanking to eating, it provides proteins and vegetable oil and is also used as animal feed (Wang et al., 2020; Liu, 1997). It is grown on all continents and associated with the pea family ‘Leguminosae’ and subfamily ‘Papilionoideae’ & its origin is in China (Liu, 1997).

Soy with a relatively high protein content (40%) after processing, removing fiber and oils is an excellent material for its incorporation into food. Incorporating soy proteins into a diet with a diet that is relatively poor in saturated fatty acids and cholesterol reduces risk of coronary heart disease. In addition to its exciting protein content, soy also contains healthy isoflavones, which are significant contributors to preventing cardiovascular disease, and fiber, which lowers cholesterol and improves glucose tolerance in diabetes. The fiber contained in soy has a positive effect on diarrhoea, constipation, and inflammation, as well as anti-inflammatory and anti-carcinogenic effects (Redondo-Cuenca et al., 2007). But there is also some risks because soy can contain antinutritivecompounds: trypsin inhibitors, raffinose, phytic acid and stachyose. Some anti-nutritional compounds are losing through technological processing (Becker-Ritt et al., 2004).

During the soy milk and tofu production, a raw material called okara is produced as a by-product. (Li et al., 2012). The production of 1 tonne of tofu produces 1.1-1.2 tonnes of okara, which in Asia can be more than 3,000,000 tonnes per year (Ohno et al., 1993; Khare et al., 1995; Li et al., 2008; Ahn et al., 2010). The okara contains aproximatelly 50 % fiber, 10 % fats, 25 % protein and low cholesterol (Table 1). Okara is primarily using as animal feed or industrial fertilizer (Ohno et al., 1996). As stated by Katayama and Wilson (2008), dried okara, a high-fiber residue left over from the production and good amino acid profiles, can be found in soy milk, which has a protein content of 20 % to 27 % by dry matter and fiber content of 52% to 58%. They enriched maize tortillas with okara and determined the amino acid profile and the alterations in the tortillas' texture and flavour at varying levels of fortification. Due to the unpleasant aroma and flavour, higher amount of fortification with okara were deemed to be unsuitable by the expert panellists who evaluated the product. There was no significant change in flavour between clasical corn tortillas and tortillas enriched with okara even when the amount of okara added to the maize flour was increased to up to 10%. In tortillas with 10% of okara addition the lysine and tryptophan content was higher, so these tortillas satisfied more than 90 % of specifications descibed by FAO. According this study, maize tortillas with okara enrichment could be a reliable source of protein for consumers.

Soy protein products are usually divided to three primary categories, based on the amount of protein. These categories range from 40 % to more than 90%. All three of the product group's fundamental soy proteins come from defatted flakes, except for total and partially defatted extruded flours. These are semolina and soy flour, as well as isolates of soy protein and concentrates (Erickson, 2015). Soy milk and tofu are only two of the many products that may be obtained from the rich-nutrient soybean, but they are also used in other ways. Production of soy milk or derived products is growing in popularity nowadays because these products are very popular as alternatives in lactose-free diet as well as vegetarians. Soy beans are rich for essential amino acids in compared to other legumes, and its digestion is better like in other legumes (Spring, 2005).

Table 1. The basic content of okara (g.100g-1 dry matter) (Van der Reit et al., 1989; Li et al., 2008; Redondo-Cuenca et al., 2008; Mateos-Aparicio et al., 2010).

Fat	Protein	Carbohydrates	Dietary fiber	Ash
8.5	33.4	3.9	54.3	3.7
9.8	28.5	5.1	55.5	4.5
8.3	15.2	4.1	42.4	3.9
9.3 – 10.9	25.4 – 28.4	3.8 – 5.3	52.8 – 58.1	3.0 – 3.7

Lipoxygenase in soy

Lipoxygenase-1(LOX-1), lipoxygenase-2 (LOX-2), and lipoxygenase-3 (LOX-3) are the three main lipoxygenases found in mature soybean seeds. Lipoxygenase isoenzymes “LOX1”, “LOX2”, and “LOX3”, which Glyma, “13g347600 (GmLox1) encode”, and “Gly Glyma, 13g347500 (GmLox2)”, and “15g026300 (GmLox3)” are mostly present in the ripe soybean seeds These compounds are responsible for berry flavour formation, especially LOX2. Lipoxygenase isoenzymes – single, double, and triple have all been found to have manufactured or natural mutations (Wang et al., 2020). Soy bean are rich for polyunsaturated fatty acids (PUFA), especially linoleic acid (C18:2), forming up approximately 50%, and linolenic acid (C18:3), which represents up 11%.

These compounds have a cis, cis-1,4-pentadiene structure which cause fast oxidation to hydroperoxides. Hydroperoxides as products from oxidation are transformed into volatile substances with unfavourable flavours (Yang et al., 2015). Whole soy flour is sensitive to oxidation, resulting in an unpleasant flavour that is difficult to disguise. PUFA and LOX are divided in the cell prior to the bean's disintegration. The PUFAs are combined and start to react, forming volatile chemicals and oxidation products , still during the soymilk manufacturing process. One of the volatile substances in soy products is hexanal, which is mostly responsible for unfavourable scent or aroma of soy foods (Wilkens and Lin, 1970).

Lines of LOX-free soybean have been developed by completely or partially removing the LOX found in soybeans thanking to breeding. This had positive effect to decrease compounds that give food its stale flavour, which may be helpful in improving sensory qualities and customer acceptance (Yang et al., 2015; Hildebrand et al., 1991). Non-bred varieties contain in soy milk the higher level of volatiles with compare to varieties made from non-lipoxygenase soybean cultivars included fewer enzymes. Tofu prepared from zero lines of LOX-2 soybeans tasted less good When compared to the same sensory panel controls. While soybeans LOX-free have lessened the distinctive flavour of various soy products, especially tofu and soy milk. LOX zero soybeans probably have a better properties during storage than traditional soybeans in terms of pH changing and the solids composition of soy milk, and also have better scent and taste . Soy milk stored for three months or fifteen months did not change, from a sensory perspective. (Yang et al., 2015).

LOX-free lines from Australia on soy meal storage stability and sensory qualities has not been determined. Two varieties of soy milk in Australia, are sold commercially, one from soy protein isolate imported from abroad and the other from whole soy – native sources. This species' availability on the market is reportedly restricted by the berry-grass flavour of whole bean soy milk (Yang et al., 2015).

Soy lipoxygenase-free

Generally soybeans without LOX or only small amounts are prefered. So, industry must go through a strenuous breeding process, which allows a technological process for genome modification called CRISPR-Cas9. In this process are modified oil profile in soybean seeds, flowering process and plants morphology (Wang et al., 2020). The three “lipoxygenase isoenzymes (LOX)”, “LOX-1”, “LOX-2” and “LOX-3”, are found in soy (Axelrod et al., 1981). The substrates for the oxidation are “*cis*, *cis*-1,4-pentadiene” processes, such as linoleic acid (18: 2) and linoleic acid (18: 3). The exercise of “LOX isoenzymes” factors into the growth side tastes marked as grassy, green, painful, astringent and hot in soy items by oxidation of polyunsaturated fatty acids. This oxidation causes the product to become unacceptable to consumers due to its unpleasant taste and aroma. The removal of individual LOX isoenzymes has a positive effect on sensory properties in all respects. “LOX-2” might be the main isozyme accountable for the development of hexanal in aqueous soy homogenates, but other LOX isoenzymes are also catalysts for these reactions. Soy milk made from soybeans without LOX-2 had better sensory properties than soy milk from soybeans lacking “LOX-1”, “LOX-3”, or both “LOX-2” and “LOX-3” (King et al., 2001). Soy milk and tofu, which were made from bred beans without lipoxygenases, had better sensory properties, including odour and taste, than soybeans with

LOX. made from LOX-free soybeans had a weaker boiled odor than soy milk and tofu made from normal soybeans (Torres-Penaranda et al., 1998). Bread prepared with soy flour lacking LOX-2 contined lower level of hexane with compare to dough made with regular soy flour or soy flour lacking LOX-1 or LOX-3 (Addo et al., 1993). Bread enriched with more than 10 % of soy enhanced the protein content and amino acid profile, also rheological properties, and the bread volume were better. Textured or extruded soy constituents increase production, decrease costs, improve cohesion, and increase moisture and fat binding. High-protein sports drinks and infant foods with flavours and sweeteners employ isolates mostly greater than 90% protein (King et al., 2001).

Soy products have a demand on the market due to their added value to foods, mainly in protein and oil content. Soybean protein and isoflavones enhance lipid metabolism by engaging low-density lipoprotein receptors (Anderson, 2003). In addition to their nutritional advantages, soy proteins are associated with lower menopausal symptoms and a fewer risk of some chronic diseases, including heart disease, osteoporosis and cancer (Lobato et al., 2011). Soy proteins also increase level of triglycerides (TG), low-density lipoprotein cholesterol (LDL-c), and total cholesterol (CHOL) (Reynolds et al., 2006). Producers of soy foods estate that soy has a positive function in soy protein's ability to reduce the risk of forming coronary heart disease. Enriched of food products like cereal bars with soy can be possible and perspective for future (Lobato et al., 2011).

Okara (soybean paste) is by-product from soy processing, especially soy milk and tofu. Okara is neutral in taste and has yellowish-white colour. During the hydrothermal processing of ground soybeans are produced an insoluble fraction. The high water content (80%) of okara causes major environmental hazard when thrown away. This by-product still has many useful and bioactive compounds attracting increasing interest in nutritional supplements. The addition of okara to products as a by-product has, in addition to nutritional positives, also positive environmental effects. In addition to its delicate taste, colourless appearance and easily digestible carbohydrates, okara is also suitable for gluten-free products processing. Cereal products like biscuits, crackers and morning cereals, are a useful source of complex and simple sugars and fiber for humans. Consumption of cookies is socially or religiously accepted. There is a wide range of options from which to make cookies and it usually has a long shelf life. Sugar, fat, and flour, mixed with other smaller ingredients are the basic ingredients for making cookies and form a cookie dough. (Ostermann-Porcel et al., 2017). Quality indicators of quality and indicators of friendliness for the consumer cookies are appearance, texture, taste and smell. Sugar contributes to the fragility, hardness, colour and volume of cookies. The volume, aeration and overall appearance of cereal products is influenced by fat (Ostermann-Porcel et al., 2017).

Biscuits belong to bakery products category as unique products with a specific texture and taste; thus, all generations commonly consume them as a snack. Cookies are tasty, but most cereal recipes use wheat flour, which results in cookies that are rich in calories and poor in fibre (Park et al., 2015). Okara, a fiber-rich by-product of soy processing, is used in biscuits for its nutritional value and sufficient material and properties, such as good quality water retention and emulsification (Park et al., 2015). Biscuits with 30 % of wheatt flour replacement with okara were not noticeably different from variant without replacement - brittleness, interaction of the water and rheological features. The appearance, texture, flavour, and water activity of soft-baked coconut-based snacks were better with adding 30–40 % wet coconut replacement increased nutritional properties and produced the very good overall sensory acceptance (Radocaj and Dimic, 2013; Grizotto et al., 2010). Dry okara is better for producing okara snacks, because fresh okara contains high level of water (about 80–85 g.100 g^{-1}), but its usage in the food industry is restricted, due to hastens the deterioration process and high costs to produce dry okara. Various substances are used for improve the texture of gluten-free cookies. Okara is used in cookies for bakery items benefits with other functional qualities, including enhanced texture, storage life and flavour. Coookies enriched with fresh okara increased fiber content, which had positive effect to rheological properites, because increase water absorption what is essential for combining additional components to produce a cookie path. Without adding water, fresh ocara water can be used to manufacture biscuits, and it also lowers the cost for drying of wet ocara (Shin et al., 2013; Park et al., 2015).

Children of various ages worldwide like biscuits since they are easy to make, might have a lot of healthy ingredients and may keep for a long time (Hawa et al., 2018; Aziah et al., 2018). Biscuits can be prepared with a various flour types, which are usually rich for sugar content and dietary fiber, which good binds water. Commercial biscuits are made from white flour, which has a lower nutritional value than wholemeal flour. Okara is a functional raw material and material with a promising use as a nutritional supplement and a basic raw material for production because it has low production costs and a high content of nutrients (fiber) and soybeans (soy milk) by-products (okara).

Dietary fiber is very important to human health from physiologically and from warding off many kinds of ailments. Okara is rich for fiber, so has many health benefits. We divide fiber into four types: “crude fiber, insoluble fiber, total fiber, and soluble dietary fiber”. Okara is very rich in insoluble fiber and has a low content of soluble fiber. Chemical or enzymatic processing, fermentation by microorganisms can turn insoluble fiber into soluble (Li et al., 2012).

Okara is rich not only for proteins but also for dietary fiber. In addition it is cheap and has a variety of sources. It has the potential for diet, especially diabetes patients. Wheat flour replaced with 25 %, 15 % and 10 % withh okara for produce noodles, stewed breads showed positive effects in flavour and quality from their conventional counterparts.

Okara is suitable for preventing diabetes, so can be used as food additives (Lu et al., 2013). Genta et al. (2002) used okara to soy candies producing to increase the accessibility for direct utilization of soy proteins and the advancement of soy foords. They results showed that probands accepted and favoured the lowest degree of ocara addition, which was found to be 18.3 % (based on 100 % composition). Results also confirmed that okara can be used as beneficial protein source for the better lifestyle of people. Production of soy-based or okara food product has been significantly negative influenced by the unfavourable odour of okara, which is referred to as "beany". Lipoxygenase enzymes oxidise unsaturated fatty acids when processed soy protein products, causing the negative odour or scent (Wilson, 1995).

Nutritional composition of okara

Okara has a lumpy structure and appearance, as most of its approximately 75% moisture is bound to fiber. Most of the okara dry matter consists of insoluble fiber cellulose and hemicellulose (40–60 %). Simple carbohydrates and soluble fiber are in a significant minority and form only 4–5% (Redondo-Cuenca et al., 2008). The poor content of simple sugars limits the efficient okara treatment by noble microbes. The content of stachyose and raffinose in okara is 1.4%, which could lead to digestive problems (bloating) in some consuments. In okara can be found also galactose, fructose, glucose, galacturonic acid, xylose, arabinose and trace amounts of mannose and rhamnose (Mateos-Aparicio et al., 2010).

Okara contains 15.2–33.4% protein in dry matter, of which the two main proteins are alkaline 7S globulin and 11S globulin (Singh et al., 2015). Although, okara protein isolates have low solubility, each of the necessary amino acids is present (Chan and Ma, 1999). Okara proteins are poorly digested because they resist the digestive enzyme pepsin and pancreatin. The low molecular weight okara peptide fraction exhibits high antioxidant activity and is highly effective in inhibiting the angiotensin-converting enzyme (Jiménez-Escrig et al., 2009). Trypsin inhibitors, which make up about 5.19–14.4% of okara proteins, may be rendered inactive with the right amount of heat (Stanojevic et al., 2013).

Okara also contains a significant amount of lipids, 8.3–10.9% in dry matter. As it is of vegetable origin, it contains many mono- and polyunsaturated fatty acids consisting of palmitic (12.3 %), linoleic (54.1 %), linolenic (8.8%), oleic (20.4 %) and stearic acid (4.7%) (Mateos-Aparicio et al., 2010). During the processing of soybeans, aromatic compounds, hexyl and nonyl aldehydes and alcohols are formed because of the reaction of unsaturated fatty acids with soy lipoxygenase and hydroperoxide lyase, which are responsible for the undesirable odour and taste of raw soy milk and okara. Mentioned enzymes in soy are denatured at temperatures above 80 °C, and thus the Chinese soybean processing method (as shown in Figure 1) produces a more bean-like okara (Yuan and Chang, 2007). Japanese-processed soy resulting tastier okara. It probably due to the lower content of trypsin inhibitor (Stanojevic et al., 2013), which is better used in food production. Interestingly, perhaps, for this reason, okara is commonly sold in Japan as a packaged product, while not in China. During fermentation fatty acid derivatives can be degraded to more desirable aromatic compounds (Vong and Liu, 2016a).

During soybean processing, about 12–30 % of isoflavones remain in the okara (Wang and Murphy, 1996; Jackson et al., 2002), especially glucosides (28.9 %) and aglycones (15.4 %), with a lower proportion of acetyl genistin (0.89%) (Jackson et al., 2002). Isoflavone glycosides can be enzymatically hydrolyzed by β-glucosidase to their aglycone forms with higher bioavailability (Izumi et al., 2000). Fermentation with selected species of microorganisms as they produce β-glucosidase can be used to increase the biological value of okara (Bhatia et al., 2002).

Legumes, such as soy, are also known for their content of antinutritional substances, which then pass into the okara. These are mainly phytates, saponins and trypsin inhibitors, which limit the use of okara in feeds (Anderson and Wolf, 1995). Here, too, it is possible to use fermentation processes by selected microorganisms, which would degrade these antinutritive substances and obtain a better digestible and usable okara (Anderson and Wolf,

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1995; Wang et al., 2014). A significant number of minerals with a desirable proportion of potassium, calcium and iron are not negligible (Mateos-Aparicio et al., 2010a; Stanojevic et al., 2014). Thanks to polysaccharides in the cell walls of soybeans and proteins, unprocessed okara also shows some antioxidant activity (Mateos-Aparicio et al., 2010b).

Transformation and valorization of okara

Soy is suitable as a source of protein in human and livestock nutrition, as its plant proteins have a relatively high biological value. In addition, from an economic point of view, these proteins are relatively cheap, helping to solve the world's feed and agronomic problems. Soybean manufacturing by-products like okara rich for bioactive peptides have anticancer, antihypertensive, antioxidant, antimicrobial and antidiabetice effects. However, these peptides need to be released from the complex matrix first, for example, by proteolytic enzymes (Agyei, 2015; Puchalska et al., 2017). Antioxidant and health effects of bioactive peptides and fiber isolated from okara have also been observed *in vitro* and in animal models (Yokomizo et al., 2002; Jimenez-Escrig et al., 2008; Jimenez-Escrig et al., 2010; Nishibori et al., 2017).

Another interesting component is isoflavones, which are extracted using alcohol for maximum yield (Jankowiak et al., 2014c). However, due to the length of the alcohol extraction, water is preferred as the extracting agent despite the decreased yield (Jankowiak et al., 2014b). Okara is rich for beneficial compounds including fibre, fat, and isoflavones, due to the low solubility prevents it from being used as a fertiliser or food additive. In the study of Orts et al. (2019), enzymatic degradation with endoproteases led to the conversion of before insoluble proteins to soluble peptides by around a half. Together with protein degradation, also before trapped isoflavones are released from the insoluble protein matrix. According to these authors extraction 63% of the total isoflavones content, especially more bioactive isoflavones–aglycones, which increase 9.12 times, and this is mainly due to the enhanced solubilization and interconversion of original isoflavones . Degradation of okara with endoproteases, which authors used help them developed a new product rich for bioactive peptides and aglycones with stronger antioxidant activity whit compared to untreated okara. By mentioned, the authors suggest that enzymatic extraction of valuable nutritional components of okara is more than suitable compared to conventional extraction (Orts et al., 2019).

Okara is very interesting nutritional raw material however, it is not widely used as a food ingredient as the fiber it is rich in is poorly soluble and adversely affects the texture and therefore, its physico-chemical properties need to be adjusted before use. Nano-cellulose technologies are very perspective for treating the okara. They increased dispersibility of cellulose viscosity, and surface area to volume ratio in the okara, thereby inactivate α-amylase activity. They also enhanced surface area of the okara and its dispersibility benefit the formation of short-chain fatty acids in the gastro-intestinal tract (Nagano et al., 2020).

Fayaz et al. (2020) showed how affected properties of okara high-pressure homogenization. In their research, the okara dispersion at a concentration of 10 g.100 g^{-1} was subjected to 1 transition at 50, 100 and 150 MPa and 5 transitions at 150 MPa. The authors found that the use of high-pressure homogenization disrupted the structure of okara particles to the form of physically stable homogenates with higher viscosity, caused mainly by the increase in solubility of okara because of the liberation of the fiber and the protein. The number of free proteins was 90% higher than in the untreated okara (Fayaz et al., 2020).

Microbial treatment of okara

Transformation of okara proteins with selected microbes provides several advantages. The conversion of high molecular weight proteins in the okara can contribute to their better solubility and usability. At the same time, various bioactive peptides and amino acids can be released and trypsin inhibitors are degraded, improving the nutritional quality of okara. However, the action of microorganisms can also reduce the content of essential amino acids. Therefore different effects of fermentation on the amino acid profile, peptide molecular weights, and trypsin inhibitory activity need to be considered. All these factors can positively or negatively affect the final product of biotransformation-fermented okara, and its general functional characteristics, including its bioactivity and solubility (Vong and Liu, 2016a).

Fungal treatment

Okara is an ideal environment for adhering to and developing tiny fungus due to its physical properties. Microscopic fungi decompose lignocellulosic biomass by secreting cellulolytic enzymes (endoglucanases, exoglucanases and β-glucosidases). There are two advantages to this, microscopic fungi thrive naturally and make the okara more digestible (Vong and Liu, 2016a).

In the experiment of Fujita, Funako and Hayashi (2004) okara was fermented with a strain isolated from soil in Osaka *Aspergillus* sp. HK-388. As a bioactive compound v after extraction with methanol, 8-

hydroxydaidzein was isolated in an amount of about 30 mg.kg^{-1} fermented okara (wet base). This substance was not observed in the unfermented okara. According to the authors, this bioactive substance was created by the biotransformation of daidzin and daidzein, which are present in the okara and have potential pharmaceutical and cosmetic applications. Wongkhalaung, Leelawatcharamas and Japakaset (2009) monitored higher amounts of monacolin K (cholesterol lowering agent) at 192 mg.kg^{-1} okara in the dry matter under optimized conditions after biotransformation with the purple-red mold *Monascus purpureus* IFRPD 4046. This substance is approved for its purpose for sale in the US and the EU (European Food Safety Authority, 2011; Childress et al., 2013).

Okara is commonly used in mixtures intended to cultivate edible mushrooms in Japan and the USA (Yamanaka, 2005; FAO, 2009). This has prompted several studies to grow polysaccharide-rich and healthy edible mushrooms. Li et al. (2016) reported that the growth of fungal fibers could disrupt the complex structure of okara, thereby releasing bioactive oligosaccharides from fiber and antioxidant peptides from proteins. The resulting product would have a synergistic effect from fungal polysaccharides and okara bioactive substances. Extraction methods very effective influence the properties of bioactive compounds. Shi et al. (2014) determined taht extraction with ultrasound extraction instead the hot water increase the yield of polysaccharides by more than two times. Further research could confirm that okara is an excellent agro-waste substrate for the extraction of fungal polysaccharides and, at the same time, biotransformation itself.

Bacterial treatment

Among the bacterial valorization of okara, most studies aimed at the fermentation process by the *Bacillus* species because they can be made extracellular alkaline proteases (Bhunia, Basak, and Dey, 2012) and they are naturally present in many fermented soybean products. *Bacillussubtilis* var. *natto* or *B. subtilis* showed higher antioxidant activity and bioactivity in okara fermentation. *Bacillus natto* is commonly used to make the traditional Japanese food Natto with a slimy texture and spicy taste, which is produced by fermenting whole soybeans. Natto is a rich source of bioactive peptides, aglycone isoflavones and fibrinolytic enzymes (Hu et al., 2010; Sanjukta and Rai, 2016). Yokota et al. (1996) observed the antioxidant activity of the extracts after replacing whole soybeans in Natto with okara. The extracts scavenged free radicals and reduced inflammation *in vivo*.

Okara fermented with *Bacillus subtilis* has also shown increased *in vitro* antioxidant activity. *B. subtilis* produces proteinases that subsequently hydrolyze complex proteins in the okara. Υ-polyglutamic acid (Oh et al., 2007), bioactive peptides (Zhu et al., 2008a) and a fibrinolytic enzyme found in Natto–nattocinase (Oh, Kim and Lee, 2006; Zu et al., 2010) also contribute to the bioactivity of okara. For the best bioactive substances extraction from fermented okara, it is important to optimize fermentation and subsequent extraction and, at the same time compare the content of bioactive substances in fermented and unfermented okara as well as the content of anti-nutritional substances found in soy and thus in okara (Mateos-Aparicio et al., 2010b).

Several authors have investigated the alpha-glucosidase inhibitory activity present in the fermented okara. Zhu et al. (2008b) compared α-glucosidase inhibitory activity on selected microorganisms in the unfermented and fermented okara using *B. subtilis* B2. The inhibitory activity of methanol extract (0.625 mg.ml^{-1}) and aqueous extract (0.313 mg.l^{-1}) was more than 90% higher compared to the very weak inhibitory activity of unfermented okara. Zhu et al. (2010) identified and purified an α-glucosidase inhibitor in the fermented suspension of okara as 1-deoxynojirimycin (DNJ). 1-DNJ and its derivatives are potential therapeutic agents in treating diabetes, HIV infection and Gaucher disease (Jiang et al., 2015). Industrial production of 1-DNJ is based on a complex biotechnological-chemical process (Vichasilp et al., 2009) and therefore, the use of fermented okara to isolate 1-DNJ can be attractive.

Cis-9, *trans*-11-conjugated linoleic acid (CLA), is also a bioactive substance that can be obtained by fermentation of okara. Vahvaselkä and Laakso (2010) first hydrolyzed the okara for 3 weeks with lipolytic oatmeal at a water activity of 0.70 and then fermented it with *Propionibacterium freudenreichii* sp as a 5% aqueous suspension. After 21 hours, 99% of the original free linoleic acid was consumed and the CLA yield was 22 mg.g^{-1} dry matter.

Yeast treatment

More research is necessary in area yeast biotransformation of okara. The research focused on the fermentation or biotransformation of okara with yeast is mainly to modifying of existing products, which can have better nutritional and sensory properties (Vong and Liu, 2016a).

For example, Rashad et al. (2011) investigated the feasibility of producing yeast-fermented okara to improve its nutritional quality. Selected yeasts *Candida albicans, Candida guilliermondii, Kluyveromyces marxianus*

NRRL Y-7571 and NRRL Y-8281, *Pichia pinus* and *Saccharomyces cerevisiae* were inoculated into okara. After solid-state fermentation, the authors observed more protein, minerals, and reduced lipids, carbohydrates, and crude fiber. The antioxidant activity found *in vitro* was 1.5–2 times higher in the fermented okara than in the non-fermented control. According to the authors, this is probably due to the action of extracellular enzymes and the metabolic activity of yeast.

Vong and Liu (2016b) investigated changes in the aroma of okara after its solid-state fermentation by 10 selected yeast species (dietary *Yarrowia lipolytica, Debaryomyces hansenii, Geotrichum candidum, Kluyveromyces lactis* and wine *S. cerevisiae, Lanchancea thermotolerans, Metschnikowia pulcherrima, Pichia kluyveri, Torulaspora delbreuckii, Williopsis saturnus*). Selected yeasts with proteolytic and lipolytic properties was used for producing od aromatic esters. By fermentation, the authors observed the removal of the unpleasant grassy odor of fresh okara, which is caused mainly by saturated and unsaturated C-6 aldehydes. These have been converted to methyl ketones and / or esters with a more pleasant aroma, especially when used by *Wiliopsis saturnus*–okara smelled pleasantly fruity and had the largest and widest range of C-6 esters. The treated okara could easily be further used or the desired esters isolated.

Shi et al. (2020) investigated the effect of solid-state fermentation of okara on the composition and selected qualitative parameters after applying probiotics and two species of yeast (*Saccharomyces cerevisiae* and *Hansenula* sp.). Various combinations and fermentation schemes were applied and the approximate composition, ability to improve selected physicochemical, antioxidant, functional and sensory properties of the okara were monitored. The results of this research show that in all applied fermentation schemes (probiotics + both yeasts), there was an improvement in the selected properties of okara, especially the bitterness. Namely application of a mixture of probiotics and both selected yeasts resulted in crude fiber content of 12.0 g.100 g^{-1}, total phenols 154 mg gallic acid equivalent per 100 g, and free amino acids 3.80 g.100 g^{-1} (dry matter). The antioxidant activity determined by the DPPH method was 776 mg Trolox equivalence per100 g and (dry matter). At the same time, the transformed okara contained more alcohol, esters, and other important aromatic substances.

Okara and its derivates as potential soil enhancers

Economic benefits can bring the recovery of biowaste and food industry by-products into organic fertilizers which can effective reduce the negative environmental impacts (Du et al., 2018). Digestates of biological origin can be used on non-agricultural land (Peng and Pivato, 2017), for example, for soil remediation after its processing into biochar or as a top layer after the closure of municipal waste landfills (Ahmad et al., 2014). In addition to fertilizer and energy properties, biodegradable wastes from the food industry could also find application in refining processes, during which valuable products rich in colorings, proteins, enzymes, and organic acids would be obtained (Hagman et al., 2018; Teigiserová et al., 2019).

Regular nutrient fortification of soil is a common agricultural practice, whether to increase soil productivity or to recultivate it. Organic matter of various origins is widely employed for these purposes (Tejada et al., 2014; Aranda et al., 2015). Used organic matter is mostly rich in organic compounds, such as residues from primary agricultural production and the food industry (Tejada & Benítez, 2014; Goswami et al., 2017; Peltre et al., 2017). Organic products and wastes have usually complicated structure and must first be before processing their nutrients fully recoverable and decomposed or transformed. However, this takes various lengths of time (Tejada et al., 2014; Franco-Andreu et al., 2017).

In recent years, stimulants have been used that are obtained, for example, by enzymatic hydrolysis of various agricultural and food by-products, such as cereal germ (Parrado et al., 2008), sewage sludge (Rodríguez-Morgado et al., 2015a), wheat distillate (García-Martínez et al., 2010), sunflower meal (Ugolini et al., 2015), chicken feathers and rice bran (Tejada et al., 2011), and others. Thanks to the process of enzymatic hydrolysis, these complex products decompose and transform into products richer in amino acids, humic acids, peptides, and polysaccharides compared to the original product. Thanks to the higher concentration of active substances, they support microbial activity in soils and help its regeneration process. For example, selected studies have concentrated on the impact of biostimulants on the degradation of polycyclic aromatic carbohydrates (Rodríguez-Morgado et al., 2015b) and pesticides (Rodríguez-Morgado et al., 2014). It was also observed a positive effect of biostimulant application on production, fruit quality and yield (Parrado et al., 2008; Colla et al., 2015; Ugolini et al., 2015; Tejada et al., 2016).

Due to the above-mentioned, to produce fertilizers and soil stimulants, it is also possible to use a by-product from the processing of soybeans–okara, which is lot of organic material. Okara is rich in protein and fiber, which, when properly transformed, can release various simpler substances (Rinaldi and Bennink, 2000). In addition to the dominant fiber and protein, okara also contains small amounts of fats, other carbohydrates and isoflavones. Due to its composition, it appears to be an interesting raw material for further processing (Redondo-Cuenca, Villanueva-Suárezand Mateos-Aparicio, 2008; Mateos-Aparicio, 2011; Galanakis, 2012; Jankowiak et al., 2014). However, okara must be processed quickly and efficiently; as it contains a high percentage of water, it perishes quickly. Although it can be preserved by drying, it is highly unprofitable. For these reasons, this by-product is currently used either as an organic fertilizer or animal feed (Rinaldi and Bennink, 2000). Hydrolysis of okara and the subsequent availability of its biologically valuable substances appears to be a promising way for its further use as a nitrogen-rich organic fertilizer, thus limiting the use of chemical fertilizers (Orts et al., 2018).

The annual droughts clearly indicate the enormous importance of water for plant growth (Barnabas et al., 2008; Pennisi, 2008; Fahad et al., 2017). Most of the water used in agriculture comes from groundwater and at the same time, this sector consumes up to about 70% of fresh water (Bourzac, 2013; de Graaf et al., 2019; Grafton et al., 2018). Usually is unsustainable in many parts of the world crop irrigation (Rodell et al., 2018). Satellite tracking has shown that, for example, in the Central Valley (the most productive agricultural region in the United States), about 30 billion cubic meters of water were lost between 2003 and 2012 (Bourzac, 2013). Climate change in recent decades has been associated with frequent droughts, leading to even greater pressure on water supplies through irrigation (Bourzac, 2013; de Graaf et al., 2019). Therefore, for long-term sustainable crop production, it is necessary to address the problems associated with water, which is essential for plants.

Despite efforts to irrigate and increase yields in crop production, most of the water from artificial irrigation either evaporates or seeps deeper into groundwater. Irrigation technology plays an important role in this, as methods such as drip irrigation reduce water losses. However, due to their financial and maintenance complexity, these systems are more suitable for growing perennial plants, especially trees (Bourzac, 2013).

Polymers are currently used in agriculture to bind water about 100 times their dry weight (Farrell, Ang and Rayner, 2013; Azeem et al., 2014; Ahmed, 2015). However, these substances, also called hydrogels, are unsustainable as they are made from petroleum. Therefore, other ecological products are being sought from which such superabsorbent materials could be made (Bashari et al., 2018). Pure cellulose-based materials are suitable as promising to produce superabsorbent hydrogels. Still, they are relatively expensive and therefore, other sources as waste and by-products are sought, such as sewage sludge and garden waste (Stabnikova et al., 2005), mulberry branches (Zhang et al., 2014) or flax yarn waste (Zhang et al., 2013).

In recent years, several studies have been conducted to produce okara-based hydrogels (Songsrirote et al., 2017). In general, studies were aimed at the gelling properties of derived products from okara (Yan et al., 2015; Yin et al., 2019). However, the emphasis in these studies was mainly on the physical properties of these products, such as their swelling ability or gelatinizing properties, and they did not address their practical use in plant growth. The research is focused mainly on developing a hydrogel from processing okara regarding current circular economy requirements.

Tan et al. (2021) developed a superabsorbent okara hydrogel that can be used as a growth aid. Hydrogel applications to the roots in a concentration of 3 and 5 % enhanced the use of the water of the selected kinds of vegetable and thus its growth. The application of this product reduced the stress of the plants due to the lack of moisture during this experiment and, at the same time, better use of water in the over-watering phases. The importance of this research lies mainly in integrating circulation into the so-called circular economy, which allows plants to manage water better, minimizes watering and the substrate retains nutrients for longer, which is especially important when growing in adverse conditions.

In a study by Zhu et al. (2020), three hydrogels with okara fixed in a total of 33% were produced by varying the proportions of monomers to crosslinking agent in the synthetic process. These were applied as a stimulant in the cultivation of the common Asian leafy vegetable Choy sum (*Brassica rapa subsp. chinensis var. parachinensis*Applying of hydrogel to plants grown in pots showed better growth and higher shoot weight associated with a better leaf area than plants without hydrogel application. In addition, seedlings supported in this way showed better seedling survival and more than an 80% increase in growth under water-deficient stress conditions after applying 2% okara hydrogel, suggesting its potential use in growing plants even in extreme conditions (Zhu et al., 2020).

Another potential way to transform and evaluate okara is to use a specific insect. Black soldier fly larvae (*Hermetia illucens* L.) are known for their greed, rapidly and efficiently devouring any organic material or waste such as coffee cake, distillery cake, municipal biowaste and many others (Rehman et al., 2017; Setti et al., 2019) and can be used in bio-transformation of okara.

Chiam et al. (2021) reported that black fly larvae could biotransform okara to frass by up to 85% of the original mass. This mass contained enough nutrients for growing lettuce and the application of additional fertilizers was necessary only after the first growth cycle. The authors further describe in their research that adding 10% frass to the soil seems to be the most suitable when lettuces of similar quality as in the control soil were grown. At concentrations of 20 % and 30 %, the authors no longer observed the desired results, as the lettuce was stunted, which was probably due to the rapid mineralization of frass from okara. The application of frass from okara also showed a slight reduction in soil microbiota compared to the control, but higher amounts of Cu, Mo, Zn and Ni minerals were not present in the pure soil. It were no detected pathogens in 10 % of okara enriched soil; therefore, the authors recommend this amount as safe for agricultural using (Chiam et al., 2021).

Orts et al. (2018) prepared okara by fermenting process and hydrolyzed it using enzymes to extract some soil biostimulants. It were significant differences in chemical composition between fermented hydrolysates, okara and enzymatic hydrolysates. They showed not only more protein, potassium, but also flavonoids and vitamin C. Profile of molecular weight of fermented hydrolysates was lowest because they include the most peptides that were synthesised from the okara proteins. Soil conducted experiment showed that both hydrolysates and okara increased phosphatase, glucosidase and dehydrogenase activity. It was also found that stimulate of dehydrogenase and phosphatase was more effectively than okara or fermented biostimulants. Biostimulants used for soils treatment also effectively influenced the concentrations of fatty acids generated from bacterial and fungal phospholipids with compared to the control and okara soils. Only dehydrogenase was dose-dependent in hydrolysate derived by the okara treatments and enzymatic process, whereas phosphatase, microbial biomass and glucosidase were dose-dependent throughout all treatments.with pesticides or heavy metals by the application of various organic matter is often used in agriculture (Tejada et al., 2011; 2014; Gómez et al., 2014). Decomposition and mineralization of organic matter release nutrients that stimulate the growth of microbiota-tolerant xenobiotics, which then degrade more rapidly. In addition, organic matter can bind pesticides, thereby reducing their soil concentration and, consequently, their toxicity (Delgado-Moreno and Peña, 2009; Kadian et al., 2012; Gómez et al., 2014). In this context, the use of okara appears to be promising in producing biostimulants for the regeneration of contaminated soil.

Orts et al. (2017) aimed at the bioremediation capacity of okara in soils contaminated by organic xenobiotics. Authors purposely contaminated soil in the laboratory conditions with insecticide (5 $l.ha^{-1}$). Pure okara and biostimulant/biofertilizer based on pure okara were added to the soil as two types of okara using pH-stat technique. Enzymatic activities (dehydrogenase, urease, *β*-glucosidase, and phosphatase) and the evolution of the chlorpyrifos in soil were studied over 80 days. The authors found that both selected soil supplements stimulated soil microorganisms and accelerated the degradation of chlorpyrifos. However, the treated okara showed a much better acceleration in insecticide degradation. Okara after treating showed a higher content of low molecular weight peptides, which were better available for microorganisms (Orts et al., 2017).

Conclusion

Okara is promising raw material rich for various bioactive compounds, which can be used as attractived ingredient in foor industry, agriculture, pharmacy and medicine. It can be useful for people and animals. Okara after various processes and treatment can in food business create an ideal circumstances that can enhance the nutritional quality of the food, especially foods with added value. It is also possible to produce healthier animal feed at a reasonable cost for the feeding of animals. Enzymes (endopeptidase, exopeptidase) enhance the protein hydrolysates which can improve the health properties to human body in the medical and pharmaceutical and medical area. There is a big potential to used okara for food and feed production, in agriculture as a biofertilizer and supplemental diets (together with medicinal herbs, mushrooms and others). This application is actual and perspective for the ecosystem and has big potential from environmental, agronomic and nutrition point of view.

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