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Background

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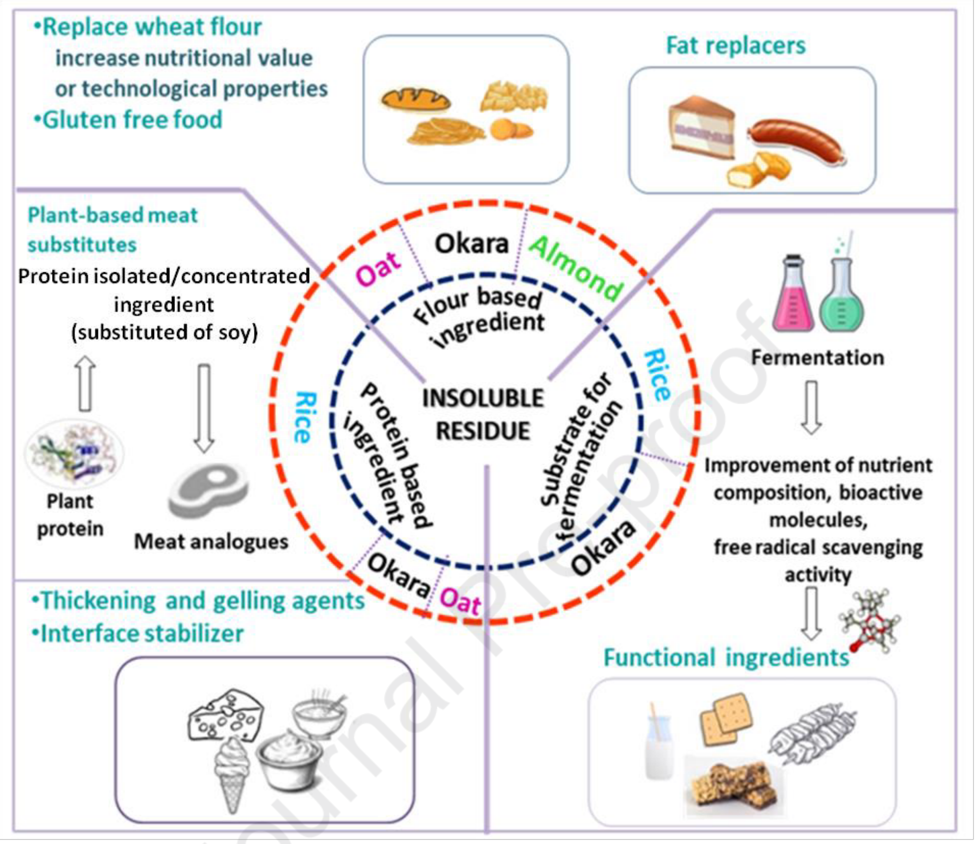
Scope and Approach

This work investigates the nutritional composition of IRs and proposes their valorization in the food industry. PBBs from soybean, rice, oat and almond were considered as the most representative examples of legumes, cereals and nuts-derived products, respectively. The IR chemical compositions and functional changes that occurs especially in the protein are discussed with regard to their use for the production of fermented bioactive compounds or as bakery- and protein ingredients.

Key Findings and Conclusions

IRs from nuts, legumes and grains have a different composition. The PBB production process yields IRs rich in fiber and fat, while most of the carbohydrates are extracted. The protein fraction behaves differently depending on its solubility the process. In the case of the cereals, proteins are significantly concentrated in the IR (+7.4 and +2.4 for rice and oat respectively). All the IRs considered can be up-cycled in food industry. IR from soybean, oat and rice seem to be suitable for the production of bioactive ingredients for functional food production; almond, soybean and oat IR can be used as flour base for bakery. IR from rice may be a good candidate to replace soy protein isolates/concentrates for plant-based meat production.

VISUAL ABSTRACT



Insoluble residues from soybean, rice, oat and almond -based beverage: landscape of the product category, chemical characterisation and valorisation in the food industry.

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Keywords: vegetal beverage; by-product; flour; plant-based meat; okara; fermentation.

1. Plant based beverage sector overview

Plant-based beverages (PBBs) are extracts of legumes, grains, pseudograins, nuts and/or seeds, and, in some cases, mimic cow's milk in appearance and consistency. PBBs that are currently on the market may be as follows: cereal-based (oat, rice, corn, spelt), legume-based (soy, peanut, lupin, cowpea), nut-based (almond, coconut, hazelnut, pistachio, walnut), seed-based (sesame, flax, hemp, sunflower), and pseudo-cereal based (quinoa, teff, amaranth).

Although PBBs are widely known as "milk," the use of this term for plant-based products is regulated or not permitted in some regions. In the EU, the term "milk" is restricted to those of almond origin (traditional product) by under Commission Decision (European Parliament and Council, 2010); therefore, the definition of plant-based beverage or non-dairy beverage is preferred.

The total plant-based milk consumption volume estimated in the considered geographies was above 8,000 million liters in 2023 (Euromonitor, 2024). A few Companies, from the US (two), Canada (two), Australia (two), France (one), and Italy (one), are the top producers in the global market.

Regionally, the largest markets for PBBs are Asia Pacific (over 8 billion €), North America (3.8 billion €) and Western Europe (3.4 billion €). The highest per capita expenditure on this product category is found in Australasia and North America (between 10.09 and 10.20 €/consumer/year) followed by Western Europe (6.20 €/consumer/year) (Euromonitor, 2024). According to Research and Markets, global sales of PBBs will reach more than $38 billion by 2024, representing a compound annual growth rate (CAGR) of +14% between 2018 and 2024 (Research and Markets, 2019). The CAGR of the global dairy alternatives market is highest in Europe, South and East Asia and its growth for the decade 2022-2032 is projected to be around 13.6% of the CAGR (Market Research Report, 2023). The growing production trend will therefore continue in the incoming years (Zenith Global Ltd, 2019), with the highest development for almond and rice beverages (+16.7% CAGR and +15.2% of CAGR between 2020-2025 respectively) followed by soy and others (hemp, pea, hazelnut, coconut, and cashew beverages) (+9.6% of CAGR 2020-2025) (Fig. SI1).

In Italy, the PBB market is divided between soy (25%) and rice (22%), followed by blended (18%), oat (12%) and almond (10%). In addition, PBBs with a different characterizing ingredient (i.e. coconut, hazelnut, spelt, walnut, cashew-based) accounted for 13% of total consumption (Angelino et al., 2020). The market is dominated by 5 companies plus the private label, which represents 25% of the total sales volume (Angelino et al., 2020; Redazione Beverfood.com, 2020).

The higher price of PBB compared to cow's milk limits the number of potential consumers. These are mainly wealthy and high-income individuals (minimum of $50,000/y in the EU) (Pritulska et al., 2021).

The main factors driving consumers to switch to a plant-based diet and purchase PBBs are their healthiness (82%), ethical and environmental (27%) issues (Euromonitor International's Voice of the Consumer Health and Nutrition Survey, 2021).

PBBs are lactose and cholesterol-free and, for these reasons, they can be a valid choice for people with lactose malabsorption (estimated at around 68% of the global population) or cardiovascular disease, respectively (Storhaug et al., 2017). Positive attributes such as calcium and vitamin content (for all beverages), fiber (for the oat beverage), and low fat content (for rice) can be highlighted with specific labelling, as allowed by regulations in different regions.

Vegan and vegetarian lifestyles prohibit the use of animal-derived foods in order to avoid cruel practices. Currently, there is no a legal definition of vegetarian or vegan food in the EU, (Carreno & Dolle, 2018). Therefore, some PBBs present the V-label certification granted by the European Vegetarian Union (Domke, 2018). However, for the overwhelming majority of consumers worldwide (42 per cent), the reason for purchasing PBBs is the restriction on eating animal products, while only 3.4 per cent declare that they consume these products because of veganism. Six percent of the consumers are flexitarians, who follow a vegetarian diet with the occasional inclusion of meat or fish, and this has gained attention in recent years (Euromonitor, 2022).

Dairy production has one of the largest carbon, water, and environmental footprints in the food sector (Sandström et al., 2018). They are generally higher than those of legumes, grains, and fruits used in the production of PBBs; the carbon footprint was estimated to average of 1.39 CO_2-eq/kg, 0.88 CO_2-eq/kg and 0.42 CO_2-eq/kg for cow's milk, soy, and almond beverage respectively. Similarly, the water footprint of cow's milk was estimated to be 3.5 times higher than that of soy beverages (Poore and Nemecek, 2018). In some cases, PBBs are labelled as non-GMO (genetically modified organisms) products, and the origin of the packaging is highlighted, such as Forest Stewardship Council® (FSC) and Carbon Trust (i.e., packaging that reduces CO_2 emissions), in order to gain claims related to environmental protection.

The production of PBBs generates liquid and solid residues. Currently, there is not much interest in reusing the liquid fraction addressed for depuration (Chen et al., 2019). The solid fraction consists of tegument and fiber, protein and ash that are not solubilized during the process. This residue is generally named here insoluble residue (IR); the residue from soybeans is widely known as okara.

The hulls and peels, when present, are a small fraction (8% -25% wet weight- w.w. of the total ingredient). They are characterized by low moisture (4-11% w. w.) and high calorific content (16.07 MJ kg^{-1} and 16.2 MJ kg^{-1}) for almond and rice hulls respectively (Silva et al., 2018). These properties are attractive for energy recovery. However, the high content of fiber and bioactive compounds such as polyphenols and vitamins suggest an alternative use in the food or feed sector, analogous to the valorization of residues from various food industries (Rahman et al., 2021; Ghany et al., 2023; Hassan et al., 2022; Soma et al., 2023; Tahir et al., 2023).

The bio/thermochemical processes of the soybean hulls have been applied to produce bio-oil, polysaccharides and oligopeptides (Liu, 2017); thanks to their metal-binding properties they were found to be useful for wastewater treatment (Li et al., 2011; Gong et al., 2008). In addition, their use as herbal medicine against headache and vertigo or for detoxification and diuretics has been reported (Fukuda et al., 2011).

IR represents a significant amount of material streams that are currently discarded as industrial solid waste or underutilized, mainly as components in animal feed, as organic fertilizer or to be converted into biofuels (biodiesel and bioethanol) or addressed to the anaerobic digestion sector (Feng et al., 2021; El-Saidy, 2011; Rahman et al., 2021).

Although several characterizations are presented for soy okara, little or no data are available for the other IRs. The existence of this gap limits both the scientific knowledge and the support for the industrial valorization of these fractions. In the present paper, the publication of the previously unavailable nutritional composition of IRs derived from PBB full-scale plant producers may be a first attempt to support the future employment of the residues. The discussion of the literature full scale production data will be addressed to identify the factors influencing the IR composition, taking into account the different source of the ingredients (legumes, cereals and, nuts) and the industrial process adopted.

Finally, scenarios for the future re-use of IRs in the food industry were described using the existing studies on okara as a reference.

2. Soybean, cereal and nut PBBs production flowchart and nutritional characterisation

2.1 Soybean, cereal and nuts PBB production flowchart

Production flowchart (Fig. 1) varies depending on the ingredients (legumes, nuts or, cereals) that require different processing steps or, in some cases, pre-treatments (Romulo, 2022).

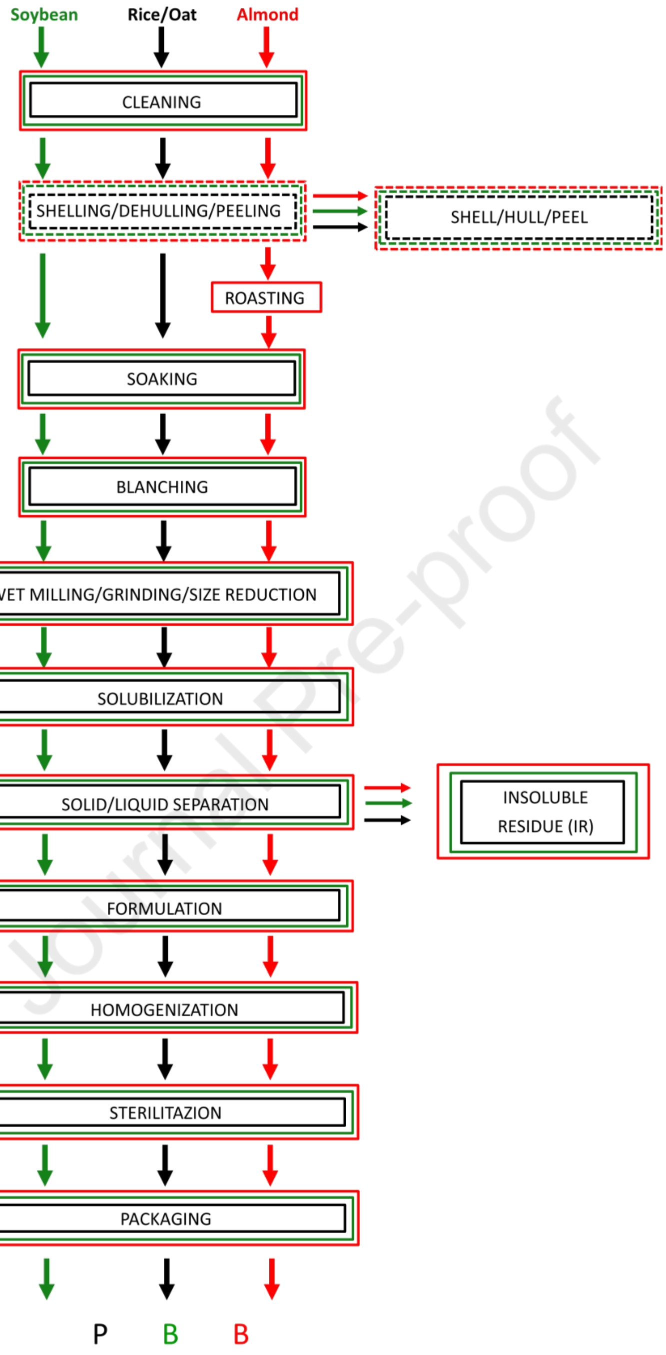

Fig. 1. Flowchart of the PBBs production from soy, rice, oat and almond. The step identified with dotted line are optional.

In general, the steps identified are:

1-Cleaning. Foreign parts are removed using a destoner, gravity and sieves.

2-Shelling, dehulling and peeling. The mechanical operation performed to increase the water absorption during the subsequent soaking phase. This step is optional for grains while nuts are usually shelled and peeled. Soybeans are preferably used de-hulled, as these fractions contain significant amounts of mono-, di- and oligo-saccharides (i.e. 5% w/w of sucrose, 5.1% w/w of arabinose, glucose and verbascose, 1.1% w/w of raffinose and 3.8% w/w stachyose) which cause flatulence when ingested. Indeed, the hulls are responsible for the beany flavor derived from the volatile carbonyl compounds, including hexanal which limits their appeal to consumers (Lusas et al., 1989).

3-Soaking. This step is primarily used to soften the texture of the ingredients and to facilitate their subsequent processing. Both temperature and duration are the key factors that affect the water absorption of the grains/nuts/beans and thus the quality of the beverage. In the case of soybeans, the use of hot water (40 °C- 60 °C) and sometimes the addition of alkalis (0.5% $NaHCO_3$ solution for 6-12 hours) reduces lipoxygenase activity, which increases protein digestibility and, the beverage flavor (Kizzie-Hayford et al., 2016). During cereal soaking, the starch swelling starts up (i.e. rice: water ratio 1:2, time=1 hour). At the end, the liquid phase is trough drained out using a filter net.

4-Blanching. Blanching inactivates trypsin inhibitors that reduce protein digestibility (Lusas et al., 1989). The use of sodium bicarbonate at high temperatures (100 °C for 10 to 30 minutes) inactivates soybean lipoxygenase which is responsible for the production of beany flavor

(Chen, 1989; Lv et al, 2011). Indeed, the increase in alkalinity improved the texture of the soy-based beverage by reducing the chalkiness due to the relatively large particles (>150 mesh) (Chen, 1989).

5-Wet grinding/milling/size reduction. Soaked grains can be ground with hot or cold water by a stainless steel disintegrator, hammer mill, pin mill or large blender to produce a colloidal solution that will pass through a 150-mesh screen. Other types of size reduction such as ultrasound, have also been tested in this processing step (Preece et al., 2017).

Feed rate, type of milling, amount of water added, temperature and, pH affect the product characteristics particularly flavor development and the presence of oxidation markers over time. Therefore, for soybeans, grinding in carried out in hot water solution above 80 °C to the inactivate the lipoxygenases (Lv et al., 2011).

6-Solubilization. The amount of water added, extraction rates, number of extraction cycles, temperatures and pH affect the extractability of the molecules and thus the nutritional composition of the beverage (Lusas et al., 1989; Quasem et al., 2009; Romulo, 2022). For cereal PBBs, alfa-amylase (0.22% w.w.) is usually added to dextrinize starch and improve the sweetness of the PBB. Other uses of protein glutaminases are described for oat protein concentrates, such as enzymatic deamidation which improves the protein solubilization. At the end of this step, the slurry is heated to 90 °C to inactivate the enzyme (Deswal et al., 2014).

7-Solid/liquid separation (S/L). The liquid phase (PBB) is separated from the insoluble phase (IR). Okara is the name of soybean IR (Dhankar and Kundu, 2020; Romulo, 2022). Removing solid particles from the slurry improves beverage stability by preventing sedimentation of insoluble particles during storage (Dhankar and Kundu, 2020). S/L separation is performed by means of a centrifuge or a decanter. The industrial setup (one or two steps, rotation speed) may differ according to the decanter size and level of recovery level of the targeted component (Flottweg, 2024). The total solids and protein contents of the liquid phase are controlled mainly through the water to bean or grain ratio. Typically, the weight of the liquid phase (i.e. raw PBB) is around 6- 10 times of the quantity of ingredient processed (Chen, 1989).

8- Formulation. Salt, sweetener and flavoring agent are added to the liquid fraction. Several flavors can be used to satisfy the consumer's preference; vanilla, cocoa, chocolate are the flavours present all around the world indeed other local flavors are also used (e.g. milk, egg, strawberry, etc. in Taiwan, fruit, vegetable, beef, yakult, honey and sesame in Japan).

Chen (1989) reported the use of 0.02% of salt and vanillin, calcium 25 mM to produce soybean PBB (bean:water 1:10). Oil, lecithin or an emulsifier are usually added to improve the creaminess and mouthfeel of PBBs (Nawaz et al., 2023). In addition, vitamins and calcium (as calcium tricitrate) may be added to increase biofortification similar to that of cow's milk.

9-Homogenization. Homogenization can be performed to break the fat into very fine and uniform droplets that remain dispersed in the solution preventing phase separation. A single pass at 2,000 to 3,500 psi at 90 °C through a dairy type of homogenizer is considered sufficient to make the final product creamier and more uniform in consistency (Lusas et al., 1989). In addition, the use of higher homogenization pressures (i.e. ultra-high pressure) reduces the particle size and increases the beverage stability, clarity, and whiteness index but does not affect the viscosity and protein stability of the PBB (Zaaboul et al., 2019).

10-Sterilization. To extend shelf life and maintain quality, pasteurization, sterilization and ultra-high temperature (UHT) treatment (121 °C for 15-20 min) are used to produce PBBs with different shelf life and storage conditions.

11-Packaging. Selecting the proper type of packaging is one of the most important decisions for successful commercial production of PBBs. For long shelf life and high stability, aseptic packaging and a cool and dry place of storage are recommended. Once the pack or bottle is opened, product storage at 4 °C is mandatory.

2.2 Nutritional composition of PBB and comparison with cow's milk

Fat in bovine milk is present in the form of globules surrounded by a phospholipid layer forming an oil-in-water emulsion: a similar two-phasic system has been described for PBBs, but the nutrient composition can be very different. In order to have a more complete evaluation, the compositions of 57 different PBBs from soy, rice, almond and oat (i.e. the most relevant for the Italian market) were collected in the mass retail channels in the area of Milan (Italy) or by consulting the website of the product companies and then compared with cow's milk full-fat, semi-skimmed and skimmed (Table SI1).

PBBs from soy and almonds had the highest fat content, comparable with full-fat milk, while those from rice and oats were similar to the semi-skimmed milk (Table SI1). PBBs were lower in saturated fat content (SFA) and higher in monounsaturated and polyunsaturated fats (almond>rice>oat) than cow's milk. Long-chain monounsaturated and polyunsaturated fatty acids are the most representative of the PBB compositions. In milk, the saturated fraction is due to the presence of short-chain fatty acids. In terms of the omega-6/omega-3 ratio, milk has a much lower value than PBBs (Walther et al., 2022).

Almond and soybean PBBs contain less carbohydrates than cow's milk. PBBs from rice and oats have a higher carbohydrate content than milk (Table SI1). The sugar and carbohydrate composition of PBBs differs from that of milk. Milk contains only lactose, a disaccharide composed of galactose and glucose, whereas PBBs contain saccharose (composed of glucose and fructose), monosaccharides (mainly glucose and fructose), polysaccharides (i.e. starch) and trace amount of dietary fiber (Walther et al., 2022). Mono-, di- and saccharides affect the glycemic index (GI) of the beverages (Fructuoso et al., 2021). PBBs from rice have a high GI, while bovine milk and oat PBBs have a medium GI. Finally, almond PBBs have a medium to low GI (Fructuoso et al., 2021).

Soybeans PBBs have a lower protein content than cow's milk (Table 1). Milk has all the essential and semi-essential amino acids able to satisfy the intake of children, adolescents and adults (Fructuoso et al., 2021), whereas PBBs have a lower ratio of essential to no essential amino acids. Considering a standard portion of beverage (200 mL), the essential aminoacid score of PBBs is of 24%, 14.2%, 3.1% and 0.4% for soy, oat, almond and rice origin, respectively, which is from -60% to -98 % than that of cow's milk (Singh-Povel et al., 2022).

3. The insoluble residues: qualitative and quantitative characteristics

Each kilogram of dry soybeans generates approximately 1.1 kg wet weight of okara (O'Toole, 1999), which is equivalent to a 25% on dry matter- d.m. basis for soybean (value calculated assuming d.m. for soybeans =89% w. w. and d.m. for okara = 20-25% w.w.). For 1 kg of oat PBB, approximately 0.85 kg of w.w. oat residue is generated, which corresponds to 17-34 % on d.m. basis is generated (Deswal et al., 2014). No further data on the amount of the IR obtained from the production of other PBBs could be found in the literature. The okara has been largely characterized from a qualitative point of view; in literature. To our knowledge, at least 16 characterizations of soy okara including compositional data macro and micronutrient content have been retrieved (Table 1). The application of the same investigative approach to the other IRs found only one characterization of IR from almond and none concerning IR from rice and oat. These gaps are filled with IR composition data obtained from a full-scale production plant of a PBB producer (Company Unigrà, Conselice, Ravenna, Italy) (Table 1). The study of the characteristics of the IRs was performed by comparing the average content of each component calculated considering the data available in the literature (Table 1). The composition of the IRs differed widely in terms of nutrient and micronutrient content: protein, fat, and fiber were the most abundant fractions when considering IRs derived from cereals (both rice and oats), IRs derived from almonds and IRs derived from soybeans, respectively (Table 1). In addition, the industrial process strongly influenced the composition of the IRs (Table 1). The most relevant effect is the high concentration of protein from cereals due to the use of hydrolytic enzymes (solubilization step). A common effect is the retention of the full amount of insoluble dietary fiber.

Knowing the amount of fiber in the raw material and the % composition of both grain/legume/nut and IR (Table 1), the contents of proteins, fats and carbohydrates in the IRs were calculated. Their sum gave the IR quantity estimation. The validity of this approach was first tested for oat and okara IRs for which data coming from PPB experimental production are available.

The estimation of the amount of okara and IR from oat were of 29.82 g d.m. 100 g^{-1} d.m. and 21.18 g d.m. 100 g^{-1} d.m. oat, respectively, in agreement with the data shown before. Thus, the same approaches applied to the rice and almond data (Table 1) resulted in IR = 22.53 g d.m. 100 g^{-1} d.m. rice and IR = 39.84 d.m. g 100 g^{-1} d.m. almond.

In order to obtain a synthetic picture of the similarity/dissimilarity between the ingredients and the IRs, Principal Component Analysis (PCA) was applied (see Methodology and Results on SI). Starting from a database composed of the main nutritional fractions (fiber, protein, fat and, carbohydrate), the elaboration identifies two significant latent variables (the Principal Components 1 and 2 - PC_1 and PC_2 respectively) (Table SI2) that allow to measure in a PC_1-PC_2 Cartesian plan the similarity between ingredients, PBBs and IRs expressed as physical distance (Fig. 2). Since each latent variable (PC_1 and PC_2) is strongly/weakly correlated with the original (Table SI3), the coordinates of the samples on the PC_1 and PC_2 gave an indication of the nutritional modification that occurred. Taking the value of the correlation coefficient $r=0.6$ as a threshold of strong correlation, the PC_1 is well correlated with fat, fiber and carbohydrate and the PC_2 with protein (Table SI3; Fig. 2). All the IRs are positioned at a higher PC_1 than the corresponding ingredient, implying an accumulation of fiber and fat (i.e. insoluble fractions) as a consequence of the reduction of carbohydrates, which are the main component of PBB (Table SI1, Table 1). A less definite behavior was observed for the protein, resulting in a lower or higher position on the PC_2 with respect to the respective constituents, implying a partial extraction in PBB or a complete retention in IR, respectively (Table 1, Fig. 2). The high temperatures applied during PBB production caused a modification of the internal hydrogen and hydrophobic bonds, resulting in the loss of secondary and tertiary structures and the exposure of amino acid hydrophobic groups (Ma et al., 1997; Zhu et al., 2018; Zhao et al., 2014; Kumar et al., 2021).

These structural modifications affected the techno-functional behavior of the protein, such as decreased solubility. This decrease is likely due to the formation of larger, amorphous structures (aggregates) and/or the formation of a three-dimensional protein-water network (gel). Since the processing conditions were very similar for all ingredients (Fig. 1), the different degrees of solubilization or modification are strictly related to the properties of the starting protein in each ingredient.

The soy protein fraction consists mainly composed of β-conglycinin (7S globulin) and glycinin (11S globulin) (Stanojevic et al., 2012). Subunits of the 7S proteins were partially hydrolyzed to smaller polypeptides that were less soluble and thus retained in the okara (Stanojevic et al., 2012). However, the high nutritional quality of the protein is maintained, as all essential amino acids except the sulfur amino acids are retained (Kumar et al., 2016; Grizotto et al., 2012).

Oat proteins are very similar to those of legumes (i.e. soy) consisting mainly of globulins followed by prolamins (avenins), water-soluble albumins, and glutelin. In the IR, the more insoluble proteins with a high glutamine and asparagine content are retained (Wrigley et al., 2017).

For the almond, the protein in IR was + 2.4 times higher than that of the starting ingredient. From a qualitative point of view, the protein is made not only composed of the insoluble part but also of a fraction of the water-soluble amandine. The full panel of essential amino acids is present with a very high intake of arginine.

Table 1- Chemical composition of the ingredient and IR of the PBB production expressed on dry matter basis.

		INGREDIENT				
		Soybean	Rice		Almond	Oat
Protein		40.78±2.24* (n=10 [1, 2, 3])	7.72±0.69 (n=5 [1,4, 5,6,7])		19.5±0.89 (n=6 [8,9])	14.87±2.7 (n=9 [1,4,10, 11])
Fat		17.16±2.50 (n=10 [1, 2, 3])	1.74±1.16 (n=5 [1,4,5,6,7])		52.29±4.62 (n=12 [8,9])	6.00±1.25 (n=9 [1,4,10,11])
Carbohydrates	g 100 g^{-1}	23.79±5.34 (n=10 [1, 2, 3])	79.78±10.59 (n=5 [1,4,5,6, 7])		5.27±1.80 (n=7 [8,9])	64.6±2.40 (n=2 [1,4])
Fiber		9.3 (n=1 [1])	2.01±1.32 (n=5 [1,4,5,6,7])		12.5 (n=1 [9])	8.1±3.54 (n=2 [1,4])
Ash		4.94±0.09 (n=2 [1, 2])	1.11±0.52 (n=5 [1,4,5,6,7])		5 (n=1 [8])	2.01±0.41 (n=2 [1,4])
Energy	Kcal 100 g^{-1}	446 (n=1 [1])	404.12±39.82 (n=4 [1,4,5,6])		579 (n=1 [9])	333±79 (n=2 [1,4])
		IR derived from				
		Soybean (okara)	Rice (n=1 [1])	*RDP***	Almond (n=1 [31])	Oat
Protein		27.42±10.11 (n=16 [12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26])	57.28±1.32	*56.3 ±8.92 (n=2 [1,28])*	14.00	35.38±0.76 (n=2 [1,32])
Fat		10.57±4.47 (n=15 [12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26])	3.31±0.66	*7.49 ±4.22 (n=2 [1,28])*	42.11	16.23±1.02 (n=2 [1,32])
Carbohydrates	g 100 g^{-1}	4.12±1.78 (n=9 [1, 12,13,14,17,18,19,23,24])	28.14±7.61	*11.4 ±2.99 (n=2 [1, 28])*		28.03±22.24 (n=2 [1,32])
Fiber		41.11 ±17.18 (n=10 [12,-13, 14,-17,18,20,21,23,24,26])	9.27±1.65	*5.7 ±2.26 (n=2 [1,28])*	40.79	10.8±7.07 (n=1 [32])
Ash		3.74±1.02 (n=14 [12,13,14,15,16,17,18,19,20,21,22,23,24,26])		*7.71 (n=1 [29])*	3.09	5.22±0.81 (n=2 [1,32])
Energy	Kcal 100 g^{-1}	82.04 ±12.09 (n=4 [15,16,18,23])	118			
Asp		7.98±4.39 (n= 4 [12,15,23,25])	19.47±3.11	*9.45±0.90 (n=3 [28,29, 30])*		
Ser		6.65±6.8 (n=4 [12,15,23,25])	10.89±1.75	*4.56±0.59 (n=3 [28, 2,9 30])*		
Asn	g 100 g^{-1}	16.65 (n=1 [23])	Data included in Asp			
Glu		13.22±7.29 (n= 4 [12,15,23,25])	36.42±5.96	*17.96±1.50 (n=3 [28,29, 30])*		

Gly	4.10±2.59 (n=4 [12,15,23,25])	8.57±0.12	*4.68±0.22 (n=3 [28,29,30])*
His	5.15±5.1 (n=4 [12,15,23,25])	4.5±0.73	*2.48±0.11 (n=3 [28, 2, 30])*
Arg	7.48±4.06 (n=4 [12,15,23,25])	15.95±2.62	*8.44±0.40 (n=3 [28, 29, 30])*
Thr	7.16±6.88(n=4 [12,15,23,25])	7.28±1.16	*3.54±0.44 (n=3 [28, 29, 30])*
Ala	7.16±7.52 (n=4 [12,15,23,25])	11.06±1.75	*5.79±0.21 (n=3 [28, 29, 30])*
Pro	15.04±22.78 (n=4 [12,15,23,25])	8.97±1.42	*8.80±2.59 (n=3 [28, 29, 30])*
Cys	6.24±7.64 (n=3 [12,23,25])	4.07±0.66	*0.73 (n=1 [30])*
Tyr	8.82±10.26 (n=3 [12,23,25])	9.9±1.58	*4.52 (n=1 [30])*
Val	6.7±5.53 (n=4 [12,15,23,25])	11.02±1.75	*5.83±0.63 (n=3 [28, 29, 30])*
Met	0.94±0.16 (n=2 [12,25])	5.19±0.83	*2.83 (n=1 [30])*
Lys	5.55±3.12 (n=3 [12,15,25])	6.65±1.06	*3.23±0.26 (n=3 [28, 29, 30])*
Ile	5.19±2.39 (n=4 [12,15,23,25])	7.98±1.29	*4.42±0.37 (n=3[28, 29, 30])*
Leu	7.67±3.97 (n=4 [12,15,23,25])	16.69±2.68	*8.38±0.20 (n=3 [28, 29, 30])*
Phe	8.02±6.68 (n=3 [12,23,25])	10.72±1.71	*5.42 (n=1 [30])*
Trp	6.97±8.24 (n=2 [23,25])	2.47±0.4	*0.85 (n=1 [30])*
NH_3	4.53 (n=1 [23])		
Gln	12.7 (n=1 2[3])	Data included in Glu	
Galactose	0.2 (n=1 [21])	Under limit detection	
Glucose	0.2 (n=1 [21])	14.3±1.4	
Arabinose	1 (n=1 [21])		
Sucrose	1.23±0.89 (n=2 [17,21])		
Maltose		7.45±0.89	

K		953.26±439 (n=5 [13, 16, 19, 21, 27])
Na		48.94±32.56 (n=5 [13, 16, 19, 21, 27])
Ca		297.46±128 (n=5 [13, 16, 19, 21, 27])
Fe		5.18±4.3 (n=5 [13, 16, 19, 21, 27])
Cu		0.56±0.42 (n=5 [13, 16, 19, 21, 27])
Mn	mg 100 g^{-1}	1.36±1.03 (n=5 [13, 16, 19, 21, 27])
Zn		1.98±1.72 (n=5 [13, 16, 19, 21, 27])
P		233.33±245 (n=2 [16, 19])
Thiamine		0.36±0.29 (n=3 [13, 16, 19])
Riboflavin		0.028±0.007 (n=3 [13, 16, 19])
Nicotinic acid		0.66±0.39 (n=3 [13,16,19])

[*] values are expressed as average ± standard deviation calculated using the literature and full scale plant data. For each value are reported the number of starting data (n) and the references used.

[1] unpublished data from full scale plant of Unigrà, Conselice, Ravenna, Italy [2] Medic et al., 2014 [3] Sharma et al., 2010 [4] Zhou et al., 2002 [5] Eggum et al., 1979 [6] Zhao et al., 2020 [7] Sitaresmi et at., 2023 [8] Moodley et al., 2007 [9] Yada et al., 2011 [10] Kourimska et al., 2018 [11] Sterna et al., 2016 [12] Zhong et al., 2015 [13] Vong et al., 2016 [14] Redondo-Cuenca et al., 2008 [15] Colletti et al., 2020 [16] Cai et al., 2021 [17] USDA [18] Gupta et al., 2018 [19] Van Der Riet et al., 1989 [20] Li et al., 2012 [21] Mateos-Aparicio et al., 2010 [22] Vong et al., 2016 [23] Guimaraes et al., 2018 [24] Matsuo et al., 1989 [25] Waliszewski et al., 2002 [26] Privatti et al., 2021 [27] Wang et al., 2010 [28] Zhao et al., 2014 [29] Zhao et al., 2012 [30] Meng et al., 2019 [31] De Angelis et al., 2023 [32] Helstad et al., 2023.

** RDP: rice dreg protein not treated

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Rice proteins are formed by glutelin, which is highly insoluble at the neutral pH as used in the case of PBB production. Despite its limited amount in the rice grain, proteins become the most abundant fraction of the IR due to an increase of + 7.4 fold. The IR from rice is very similar to the rice dreg protein (RDP), a more studied residue from the production of the starch syrup from rice (Meng et al., 2019) (Table 1). IR showed a higher content of essential amino acids than RDP, except for Lys, which is close to the minimum nutritional requirement.

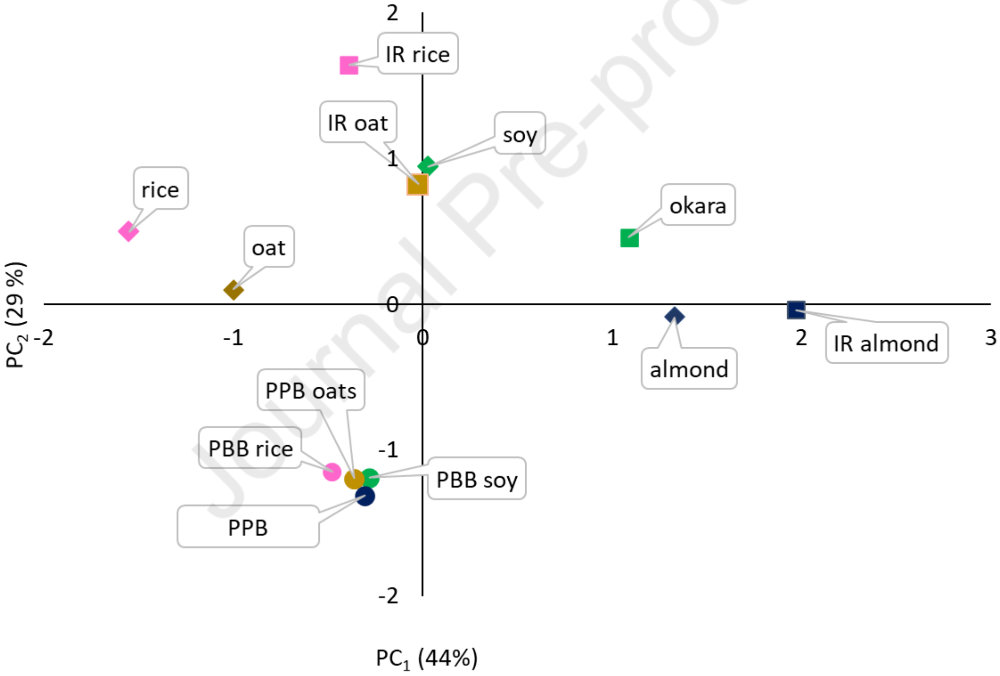

Fig. 2. Evaluation of the similarity among ingredients, PBB and IR as PCA plot.

4. The re-use of Insoluble residue from PBBs in the food industry

In recent years, IRs from PBB production have received global attention as upcycled ingredients or for technological purposes in the food industry (Guimaraes et al., 2018). Most of the literature has considered the reuse of the okara (Moraes et al., 2016). However, studies on the reuse of the residues from the production oat, rice, and almond beverages are very scarce. Bartkiene et al. (2021) suggest the use of 20% fermented almond IR or 15% fermented oat IR to improve the quality and nutritional value of wheat bread. In a study by De Angelis et al. (2023) almond IR was used at different percentages to replace wheat flour in the preparation of biscuits (See Part 4.2). Okara is used in its original form or processed into flour and subsequently used as an additional ingredient to improve the nutritional and functional properties of foods or as an additive and binding agent in beverages, bread, biscuits, cakes, sausages (Lee et al., 2021; Moscoso-Ospina et al., 2023). Okara is used as a raw material in the production of the food additive E 426 soybean hemicellulose (EFSA, 2017).

To our knowledge, industrial use of IR is limited to a few examples: in the USA, okara is used to produce flour as an ingredient in baked and flour-based production (e.g. cookies) (https://www.renewalmill.com/). An Italian company produces a flour from oat and rice IR (https://www.packtin.com/). To preserve the flavors and bioactive compounds naturally present in the ingredient, the flour is obtained through cold drying processes. A Swiss start-up has developed an okara-based burger (https://luya.bio).

The previous examples have highlighted the most interesting uses of IR in the industry. This work proceeds with a focus on the proposed use of IR as a bakery and protein ingredient as well as a fermentation feedstock for the production of bioactives.

4.1. The use of IR as feedstock for fermentation

Various studies have reported that the health benefits and nutritional quality of okara are often enhanced by fermentation with bacteria and fungi among which yeast is commonly used (Table 2). The potential applications of fermented okara as a functional ingredient have been evaluated, describing the effects and bioaccessibility, as well as its prebiotic properties (Mok et al., 2019; Sitanggang et al., 2020).

The enzymes secreted by microorganisms during fermentation can hydrolyze complex macromolecules such as fatty acids, proteins, and fibers into smaller and more soluble nutrients (Mok et al., 2019).

Proteolytic activities yielded oligopeptides and free amino acids characterized by improved properties such as the oil or water holding capacity (Hu et al., 2019).

Antioxidant activity was measured for essential amino acids (Ichikawa et al., 2022), indeed the increase of glutamic acid improved the okara of umami flavor.

Regarding lipids, the enzymatic conversion by lipases increases the content of free fatty acids, especially unsaturated ones such as linoleic and oleic acids, which have various health benefits (Mok et al., 2019).

Among the bacteria, *Bacillus subtilis*, and specifically B. subtilis WX-17, is a microorganism of interest due to its ability to degrade macromolecules and increase antioxidant activity. This can improve the overall nutritional profile, particularly by increasing the amino acid and fatty acid content and antioxidant activity.

Lactic bacteria (*Lactobacillus acidophilus, Lacticaseibacillus rhamnosus and Pediococcus acidilactici*) have been shown to be the most promising for the valorization of okara (Saadoun et al., 2021). Indeed, improvements in the nutrient composition, metabolic compounds, and free radical scavenging activity as well as aromatic fraction and bioactive molecules such as polyphenols have been observed (Gupta et al., 2018; Saadoun et al., 2021).

Mold metabolism improves the digestibility of fiber in addition to producing functional compounds (Feng et al., 2021). In particular, *Rhizopus oligosporus* and *Aspergillus oryzae* are commonly used in various foods to increase soluble fiber content and to improve nutritional and sensory properties (Lee et al., 2021).

Yeasts such as *Saccharomyces cerevisiae* improve the nutritional quality by increasing protein, carbohydrate, or total phenols of IR (Mustapha et al., 2021). Although the literature does not describe the fermentation of other IRs, the presence of similar characteristics and the use of corresponding ingredients as a substrate for fermentation suggest similar effects and the possibility of improving the IRs reuse as the source of fermented compounds and /or ingredients.

Using RDP as a reference for IR from rice, the use of *Aspergillus niger* (Liu et al., 2021) or via lactic acid bacteria (*Pediococcus pentosauceus* L11) (Chen et al., 2019) was attempted and obtained many small molecule active peptides with physiological and biological functions were (Table 2).

Table 2. Examples of fermentation of okara and RDP.

Feedstock	**Microorganism**	**Fermentation conditions**	**Functionality**	**Reference**
Okara	*L. plantarum*	10^7 CFU/10 g okara, 30°C, 48 h	Improved nutritional composition	Gupta et al., (2018)
RDP	*Pediococcus pentosauceus* L11	$3.6*10^9$ CFU/mL, 37°C, 48 h	Anti-hypertensive effect	Chen et al., (2019)
		Fungi		
Okara	*Rhizopus oligosporus*	7g/100 g okara, 30 °C, 24 h	Improve of nutritional, physical, and sensorial properties	Lee et al., (2021)

RDP	*Monascus purpureus*	5.85 log cfu/g, 30 °C, 7 days	Production of monacolin K, a cholesterol lowering agent	Japakaset et al., (2009)
	Aspergillus niger	28 °C, 7 days	Active peptides (F2ds) with antioxidant activity	Liu et al., (2021)
		Yeast		
Okara	*Saccharomyces cerevisiae*	1.25% (w/w, based on okara weight basis), 32 °C, 20 h	Dough and bread, obtaining a final product with improve of characteristic	Mustapha et al., (2021)

4.2. *The use of IR as bakery ingredient*

Several studies have demonstrated the use of okara flour in the production of biscuits, cakes and other bakery products (Table 3) (Waliszewski et al., 2002; Momin et al., 2020). The most common applications of IR as a flour derive from its fiber and protein content and include its use as a replacement for wheat flour as an emulsion stabilizer and fat replacer.

Fiber greatly influences the food's capability to retain a high amount of water. This characteristic is suitable for the production of baked food that needs hydration or viscosity development (Sanchez-Zapata et al., 2009); however, excessive fiber adversely affects sensory properties, resulting in a fibrous or gritty texture or, on the contrary, crisp or chewy due to the exaggerated water-holding property (Hazen et al., 2006).

The proteins of okara can react with the gluten of wheat flour. The formation of disulfide bonds improves the firmness, cohesion, and chewability of okara-wheat bread (Mohamed et al., 2006).

Other studies have shown a change in texture and cooking properties due to the formation of the okara protein-starch complex, which slows down starch digestibility and reduces the glycemic index (Xie et al., 2023).

The use of almond IR and oat IR (range of use 5%-20%) was tested as an ingredient of cookies and bread (De Angelis et al., 2023; Bartkiene et al., 2021). Almond IR-based flour had good water and oil adsorption capacities (Table 3), then the biscuits formulated with almond IR-based flour showed better lipid quality than those sunflower oil.

Table 3. Examples of use of IR as flour-based ingredient.

Typology and amount of IR	**Main flour**	**Aim**	**Effect**	**Reference**
Okara 5%	Wheat	Improve physiochemical, protein and sensory quality of noodles	Nutritional quality improved; cooking time decreased; general acceptability	Okpala et al., (2016)
Okara 5%-15%	Gluten-free	Increase the moisture content, thickeners, chickpea vs soy okara	Difference between chickpeas and soy okara. Sensory score for moistness higher than control	Lian et al., (2020)
Okara 4% best biscuits acceptability	Wheat	Effect on the physicochemical, nutritional, textural, and sensory attributes of biscuits	More moisture, protein, fat, ash, fiber and genistein content	Momin et al., (2020)

10% okara flour	Rice	Increase of nutritional and functional aspect in gluten free product	Improvement of nutritional and viscoelastic properties	Tridtitanakiat et al., (2023)
Okara 10%-20%	Rice	sizes effects on the characteristics of gluten-free layer cakes	10% okara flour samples presented most homogeneously bubbles distribution. It is an alternative to be incorporated in gluten-free cakes formulations. High fiber and protein contents	Ostermann-Porcel et al., (2020)
Almond insoluble residue 15%	Wheat	Improve nutritional value	More protein and fiber content. Better lipid quality	De Angelis et al., (2023)
Almond insoluble residue 20%; oat insoluble residue 15%	Wheat	Improve nutritional value	Better nutritional value and quality parameters	Bartkiene et al., (2021)

4.3. The use of IR as protein ingredient

4.3.1. Current employment

Residues from PBB production have a significant protein content (Table 1) and a good nutritional value in terms of essential amino acids. In addition, the modification that has occurred confers unique structural characteristics that are preferable to the use of raw ingredients for some technologically specific purposes (Ma et al., 1997).

With respect to the crude nature of the protein of the ingredient, those of the IR had a high degree of hydrophobicity that improved the dispersibility, emulsion stability, gelling, foaming, and fat absorption capacities (Ma et al., 1997). However, the only data available regarded the use of the okara whilst the characteristic of the protein of the other IR, above all, the technological properties were scarcely investigated (Kumar et al., 2021).

The simpler use was raw, i.e., without treatment; the raw okara, often named “pulp,” is used for fermentation to obtain food in Asian culture as an ingredient to produce alternative meat-vegetal products as a substitute for soybean (Oliveira et al., 2016).

This may be due to the fact that okara proteins have the appropriate charge to induce protein-protein interactions, promoting the formation of films at the air-water interface and consequently, increasing foam (Molina and Wagner, 2002; Sim et al., 2021). The increased presence of polar groups and the formation of film around oil droplets leads to improved emulsion stability. In addition, an unfolded protein conformation can result in better gelling properties and improved foam stabilization.

The use of the soybean as a protein-based ingredient required hydrolysis pre-treatment with mild acid or enzyme to reduce the unpleasant chalky flavor. From this point of view, the okara has better sensory characteristics since the treatment performed during the bleaching step resulted in improved flavor.

Based on the qualitative and quantitative composition of the other IR, that of oat was more similar to the okara, so a similar re-use as an ingredient for the production of beverages, desserts (ice cream, cakes, and mousses) sauces, and soups was suggested (O’Toole, 1999; Privatti et al., 2021).

4.3.2 The use of IR as ingredient for plant-based-meat analogous production

The commercial market for plant-based meat analogous market will increase (CAGR of 2022-2027 of 14.7%) (Markets Research Report, 2022) in response to consumer demand and the sustainability of future food supplies (Sanchez-Sabate et al., 2019).

Currently, production is primarily based on concentrated or isolated protein fractions from soy, pea, and rice due to their availability and cost (Maningat et al., 2022).

Various processing and physicochemical approaches are being explored to create potential plant-based meat structures to replace the texture of real meat (McClements et al., 2021). One of the most main challenges is the ability to convert the plant protein, which has a globular native structure into fibers to simulate muscle anatomy and to obtain the characteristic textural attributes of the meat mainly due to the fibrous structure of the protein.

The process was based on denaturation and unfolding, which caused the crosslinking of native chains to form filamentous structures characterized by gelling and emulsifying properties (Nishinari et al., 2018).

Zhao et al. (2014) studied the RDP as an alternative to the soybean as an ingredient in several food productions. Its use gives satisfactory results from a technological point of view especially the highest thermal stability after industrial processing. In addition, the rice protein is hypo-allergic compared to many other vegetal proteins, and is therefore used to formulate foods for infants and people with gluten-allergy (Fiocchi et al., 2003). The similar composition of the IR of rice and RDP suggested the possibility of using it for this kind of production. Moreover, in the case of cereals, especially rice, the enzymatic hydrolysis step augmented the insolubility, gelling, and aggregation degree of the protein. These improvements are considered suitable to propose as an alternative font to obtain isolated or concentrated protein fractions for meat analogous production (Fig. 3) (Kumar et al., 2021; Zhao et al., 2014).

On the other hand, the other IR has a lower protein content which discourages the use of IR from rice (Table 1).

Okara has been used as a feedstock for fermentation to obtain ingredients for meat analogous production (see Part 4.1). In a recent publication, Razavizadeh et al. (2021) used a *lactobacillus* strain as a pre-process to improve the sensory and structural properties of the okara for this kind of production. According to the Authors, 6% of okara in the formulation improved sensory and textural properties, by increasing juiciness and reducing hardness respectively.

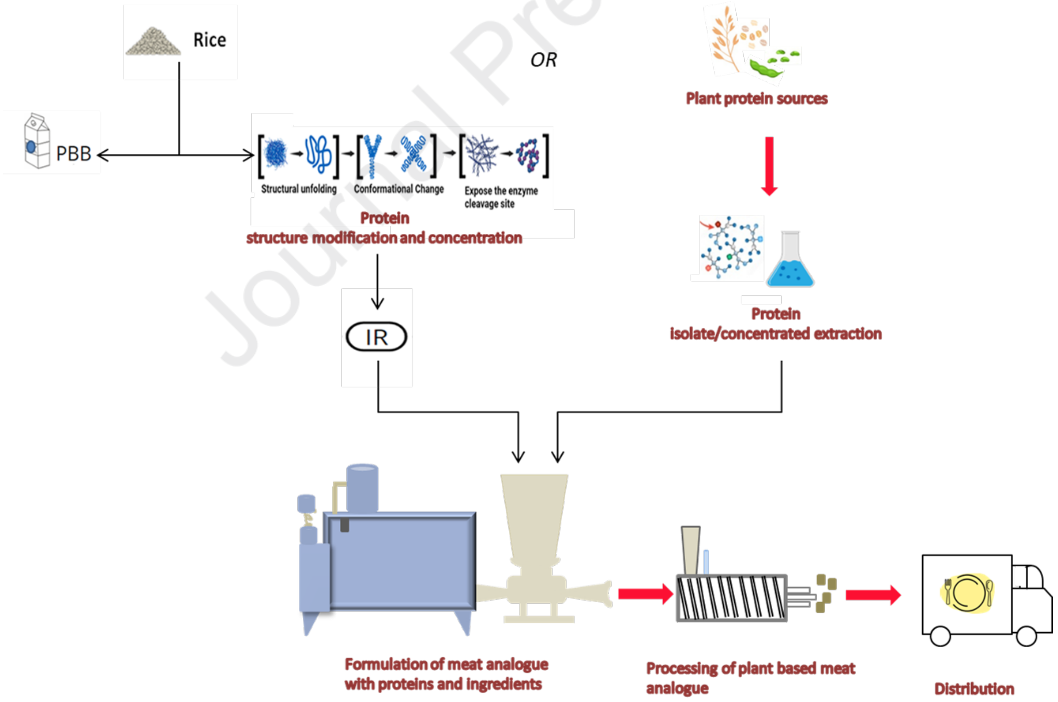

Fig. 3. Integration of the IR from rice in the meat analogous production system.

5. Conclusion and outlook

The amount of IRs from PBB production ranged from 20 to 40 g d.m. 100 g^{-1} d.m. of the starting ingredient. The origin of the ingredients and the PBB production process influenced the composition of the resulting IRs. The main components found in IRs are fiber, protein and fat. The process affected the composition and technological properties of the macromolecules, especially the proteins. Future profitable uses of IRs in the food industry are as a substrate for fermentation, as a bakery and protein ingredient with some specific uses for the different IRs. Fermentation of okara and IR from rice allows to obtain ingredients with improved functionality, bioactivity and flavor. Okara, together with IR from almonds and rice, can be used as flour in bakery products. Okara and IR from cereals have a high protein content and can be used as a source of protein-based ingredients with a technological purpose (thickeners and gelling agents). IR from rice shows promise as an alternative for soy isolated/concentrated protein in the production of meat analogues.

Author contribution statement

Conceptualization and methodology B.S., M.E. Writing—original draft preparation, B.S., M. E., T. C., E. L., M. R., C. N., M. R., M. R., A. P., C. P., D. P., S. S.; Investigation and data curation: M. E., D. B., T. C., E. L., M. R., C. N., M. R., M. R., A. P., C. P., D. P., S. S., M. T.; Review, editing D. B., M. T., D. P., A. S., B. S.; Visualization: M. E., C. N.

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Conflicts of interest

The authors declare no conflict of interest.

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Insoluble residues from soybean, rice, oat and almond -based beverage: landscape of the product category, chemical characterisation and valorisation in the food industry.

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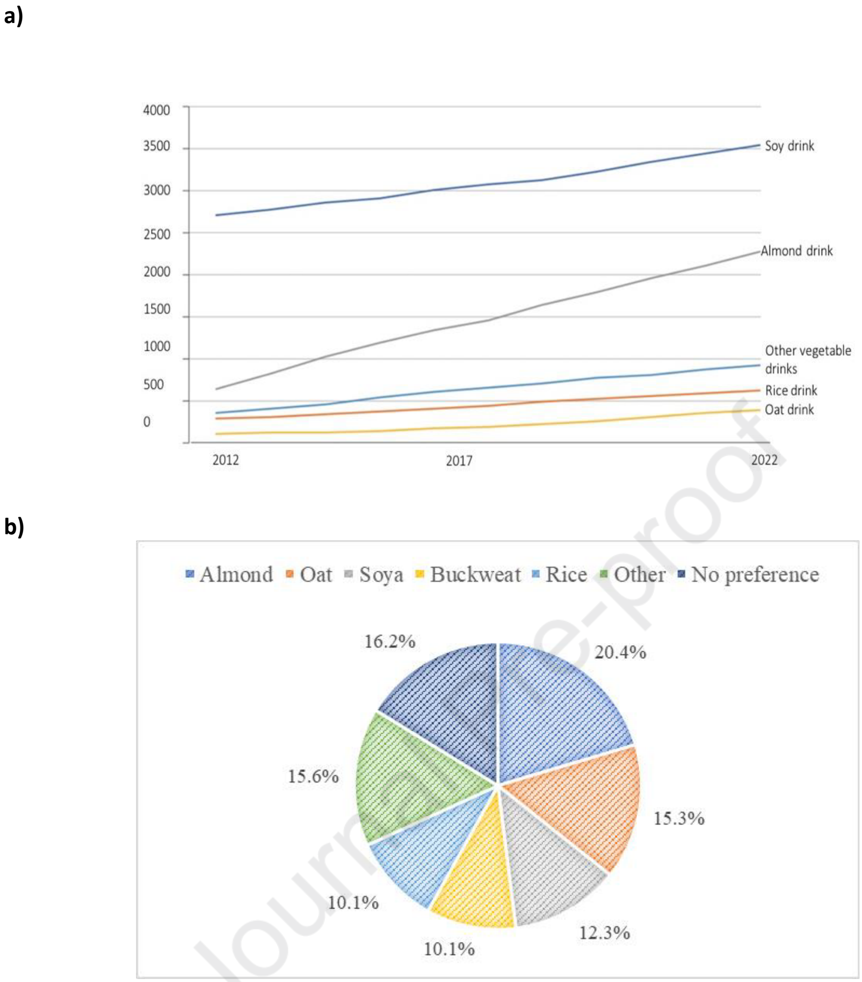

Fig. SI1. a) Global production volume in million liters (Source: Zenith Global, Ltd 2019); b) Consumer preferences of plant-based milk analogues (Source: Pritulska et al., 2021).

Table SI1- Macronutrient composition of 100 g of soy, rice, oat and almond beverages and cow's milk.

Nutritional value	**per 100g**	**Bovine milk (no-skimmed)**	**Bovine milk (semi-skimmed)**	**Bovine milk (skimmed)**	**Soy beverage N=20**	**Almond beverage N=10**	**Rice beverage N=10**	**Oats beverage N=17**
Amount (%)	%	100	100	100	4.7-13.1	2-5	8-17	8.7-16
Energy (Kcal)	Kcal	65-66	47-48	34-37	39-69	12-41	26-68	37-61
Proteins (g)	g	3.2-3.4	3.2-3.4 [a]	3.2-3.4	2.1-5 [a]	0.3-0.8	0-0.5	0-1.2
Fat (g)		2.4-3.6	1.6 [a]	0.1-0.5	1.1-4.4	1-2.5	0.8-1.4	0.7-3
SFAs		2.4-2.6	1.1	0-0.07	0.1-0.6	0.1-0.3	0-0.2	0-0.4
mFs		n.r.	n.r.	n.r.	0.3-1.1	0.4-0.7	0.3-0.4	0.4-0.6
uFs		n.r.	n.r.	n.r.	0.7-2.7	0.2-0.5	0.3-0.5	0.3-0.6
Fiber		0	0	0	0-1.3	0.1-0.6	0-0.2	0-1.5
Carbohydrates (g)		4.9-5	4.9-5 [a]	4.9-5	0-7.9 [a]	0-5.4 [a]	3.7-14 [a]	5.8-10
Sugar		4.9-5	4.9-5	4.9-5	0-7.6	0-4	0-9.1	0.7.5
Ca	mg	120	120	120	120	120	120	90-120
D		n.r.	n.r.	n.r.	0.00075-0.0015	n.r.	n.r.	n.r.
B1		n.r.	n.r.	n.r.	0.00017	n.r.	n.r.	n.r.
B2		n.r.	n.r.	n.r.	0.00021	n.r.	n.r.	n.r.

B12	n.r.	n.r.	n.r.	0.00038	n.r.	n.r.	n.r.
E	n.r.	n.r.	n.r.	0.0018	n.r.	n.r.	n.r.

The data reported were collected in the large scale distribution center of the area around Milan (north Italy) and on the web-site of the major producers of PBB.

n.r. not reported

Methodology and results of the Principal Component Analysis

In order to evaluate synthetically the similarity among ingredients, PBBs, and RI the Principal Component Analysis (PCA) was carried out.

Starting from a dataset made by the main nutritional fractions (protein, fiber, carbohydrates, and fat), the PCA approach allowed to reduce their number synthetized on latent variables, and the PC able to contain the information of the starting ones. The size of each component was identified by the eigenvalue: the earlier (and more significant) the component, the larger its size (Tabachnick and Fidell, 2001). Only the PCs with eigenvalues >1 were retained (Kaiser, 1960). Moreover, the relevance of each PC was evaluated in terms of starting variables well retained (expressed as variable vs. PC correlation index higher than 0.6 or lesser -0.6) (Scaglia et al., 2008). All statistical analyses were performed using the SPSS 29.0 package (IBM SPSS Statistics, Chicago, IL).

Results

Table SI2. Values of the eigenvalue ad degree of variance explained

Component	eigenvalue	% variance	% cumulative
1	1.779	44.483	44.483
2	1.179	29.467	73.950
3			
4			

Table SI3. Matrix of PC: correlation coefficient of the starting variables vs the significant components

	PC	
	1	2
fat	0.805	0.165
fibre	0.788	0.330
carbohydrates	-0.695	0.508
protein	0.162	0.885

Method of rotation: VARIMAX

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Highlights (3 to 5 bullet points, maximum 85 characters, including spaces, per bullet point).

- Insoluble residues (IR) are by-products of plant-based beverages production
- IR valorization is scarcely investigated, with the exception of soy okara
- Okara and IR from oat and rice are suitable to produce bioactives by fermentation
- Okara and IR from almond and oat are suitable for bakery or as flour ingredient
- IR from rice is suitable as an ingredient in analogous meat instead of soy protein