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Brown rice and pulses for the development of shelf-stable and low glycemic index ready-to-eat meals

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ABSTRACT

Shelf-stable low glycemic index ready-to-eat (RTE) risotto meals (in pouches) containing brown rice and pulses (recipe A = chickpeas; recipe B = lentils, and beans) were developed, stored for 12-months at room temperature, and characterized over time. RTE meals were heated in microwave (replicating home consumption procedure), and analyzed for *in vitro* starch digestibility, textural attributes, and consumer acceptability. Digestible starch fractions were similar in the formulations during storage, and *in vivo* testing demonstrated low glycemic indexes (recipe A = 43.5 ± 6.8; recipe B = 31.8 ± 6.5) for the two meals. Hardness of risotto components increased during storage and microwave heating did not fully recover textural attibutes characteristic of the fresh product. Consumers' (50) acceptability remained high (>5.5 out of 9) until the end of storage. This study demonstrates brown rice with pulses can be used for developing stable and accepted ready-to-eat meals having low glycemic indexes.

1. Introduction

Ready-to-eat (RTE)[1] meals are becoming popular among consumers over the past decade because of their convenience and fast preparation (Chaffee et al., 2022; Choi, 2022). In the production of RTE meals, rice (*Oryza sativa*) is gradually becoming a popular ingredient (Yu et al., 2017) for several reasons. It represents a staple carbohydrate-rich source for most people all over the world (Gondal et al., 2021). It meets the needs of time-challenged consumers (Patindol et al., 2007). It is easy to prepare, and it has a long aroma retention after cooking (Fitzgerald et al., 2009). White rice (WR) is commonly preferred over brown rice (BR) for the production of retorted RTE meals because of its sensory attributes (Gondal et al., 2021). However, the use of BR could lead to great benefits from a technological and nutritional point of view (Saleh et al., 2019). The presence of the outer shell, which makes kernels less prone to thermal damage and rupture (Mohan et al., 2017), represents a technological advantage in producing RTE meals. From the nutritional side, BR, in which only the outer husk is removed, is richer in vitamins, minerals, fiber, proteins, and bioactive compounds than polished rice (Dinesh Babu et al., 2009). Furthermore, another important reason for using BR instead of WR is related to its glycemic index (GI) which is reported to be lower than that of white rice (i.e., 73 ± 4; Atkinson et al., 2008). The lowest GI values of BR have been reported for boiled or steamed BR (68 ± 4 and 50, respectively) (Atkinson et al., 2008; Shobana et al., 2017), although, as seen for other foods, GI values of BR depend on the varietal type, cooking methods, extent of gelatinization, expansion upon cooking, and processing. Several studies reported that consuming BR instead of WR can reduce the glycemic response contributing to lowering the risk of type 2 diabetes (Malik et al., 2019; Yu et al., 2022). For example, Sun et al. (2010) reported that replacing a 50 g/d intake of WR with the same amount of BR was associated with a 16 % (95 % confidence interval, 9–21 %) lower risk of type 2 diabetes.

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[1] Abbreviations: BR, brown rice; CATA, check all that apply; FSG, free sugar glucose; GI, glycemic index; RAG, rapidly available glucose; RDS, rapidly digestible starch; RS, resistant starch; RTE, ready-to-eat; SAG, slowly available glucose; SDS, slowly digestible starch WR, white rice.

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Besides BR, pulses like chickpeas, lentils, beans, or peas can be used to complete RTE meals nutritionally. Pulses are rich in proteins, containing approximately 21–25 % protein (Singh 2017), dietary fiber, minerals, and vitamins (Keskin et al., 2022). Therefore, using BR in combination with pulses could end up in a meal with health benefits, potentially lowering the glycemic response, and thus helping to prevent insulin resistance and type 2 diabetes (Mir et al., 2020; Mohan et al., 2014; Singh et al., 2021).

Shelf-stable RTE meals are required to undergo a sterilization process to guarantee their microbiological safety and their stability for long storage times at room temperature. Different technologies can be used from the most common thermal treatments (Yu et al., 2017) to the newest ultrasounds- (Chen et al., 2020) and microwave-assisted thermal sterilization (Patel, 2020). Among them, autoclaving was found to be the most effective method for stabilizing the rice bran for long-term storage (Kim et al., 2014), and a cost-effective process to prepare retorted rice (Kim et al., 2020).

A great challenge in the production of shelf-stable RTE meals is to maintain sensorial acceptability during storage. Particularly, in products containing starchy ingredients (i.e. with a minimum starch content of 50%) such as rice and pulses, one of the major modifications during storage concerns the texture characteristics due to, primarily, starch recrystallization. This phenomenon depends on several factors like time, temperature, moisture content, and the presence of other food components (Chang et al., 2021; Keskin et al., 2022). Starch retrogradation brings to the formation of resistant starch (RS), the fraction that is not hydrolyzed during digestion. RS causes the hardening of the product (Lima & Singh, 1993), and it can be partially reversed through simple thermal treatments (Federici et al., 2021). The presence of a certain amount of RS has a positive health effect related to the possibility of lower GI of a meal (Hoover & Zhou, 2003; H. Zhang et al., 2016). In addition to textural modifications, flavor deterioration might occur in retorted BR due to lipid oxidation and loss/changes of volatile compounds leading to a premature end of the shelf-life (Yu et al., 2017).

Despite nutritional benefits, BR consumption remains limited mainly due to its sensory attributes, poor appearance, long cooking time, and lack of awareness about its nutritional value by consumers (Gondal et al., 2021; Mohan et al., 2017; G. Zhang et al., 2010). Therefore, the development of palatable RTE meals containing BR and healthy ingredients could help to foster its consumption. This study aimed to develop two healthy RTE meals containing BR and pulses formulated as risotto using a cooking process in pouches. The RTE meals were analyzed during 12 months of the shelf life in terms of nutritional properties (i.e., *in vitro* starch digestibility), quality attributes (i.e., texture), and consumers' acceptability. The low glycemic characteristics of meals were confirmed in humans.

2. Materials and methods

2.1. Risotto recipes and production

BR of Roma variety was provided by Grandi Riso S.p.A. (Ferrara, Italy). Chickpeas (*Cicer arietinum*), borlotti beans (*Phaseolus vulgaris*), and lentils (*Lens culinaris*) were purchased from a organic producer (Podere Pereto, Rapolano Terme, Siena, Italy) and rehydrated overnight. Mushrooms (*Agaricus bisporus*) were purchased fresh from Società Agricola La Quercia di Pretto Enrico e Lisa S.n.c. (Vicenza, Italy), peeled, and cut into slices just before meal preparation. Onion (*Allium cepa*), rosemary (*Salvia rosmarinus*), sage (*Salvia officinalis*), and garlic (*Allium sativum*) were purchased fresh at a local market. Mushroom (*Boletus edulis*) powder was purchased from a local market. Extra virgin olive (EVO) oil was purchased from Coppini & Figlio S.R.L. (Parma, Italy). All vegetable ingredients were washed to remove eventual residual dirt, gently centrifuged with a manual spinner to remove excess water, and minced just before meal preparation. At first, six risotto recipes were developed and pre-tested to identify the two ones that were preferred by consumers based on unformal sensory evaluation. The two selected recipes, i.e., recipe A and recipe B (Table 1), were developed and the relative retorted pouches were produced by DelSanto company (Padua, Italy). All ingredients (250 g) were put into aluminum pouches (composite packaging, 406735, Goglio S.p.A., Milan, Italy) with water (60% on a dry basis). Pouches were sealed, cooked, and sterilized in a horizontal autoclave at 118 °C for 35 min, as previously described (Federici et al., 2021). The pouches were cooled down and stored for 12 months at room temperature (20 °C). The RTE meals were prepared as "risotto" where at the end of cooking all the water was absorbed by the ingredients.

2.2. Risotto safety assessment

Risotto microbial safety was verified by assessing sterility of three pouches by microbiological analysis during storage at 0, 3, and 12 months. Twenty-five g of the pouches content was aseptically transferred in a stomacher bag (Stomacher® 400 Classic bags with filter), containing 9 parts (w:v) of Ringer solution (Oxoid, Basingstoke, UK) and homogenized for 3 min using a stomacher (Stomacher Bag Mixer 400P, Interscience). Decimal serial dilutions were done in Ringer solution and plated on Plate Count Agar (PCA, Oxoid, Basingstoke, UK) to measure total mesophiles and spore-forming bacteria counts, while Yeast Extract Dextrose Chloramphenicol (YEDC, Remel Lenexa, USA) was used for yeasts and molds count, and Brilliance™ *Bacillus cereus* Agar Base (Oxoid, Basingstoke, UK) containing Brilliance™ *Bacillus cereus* selective supplement SR0230E for *Bacillus cereus* count. PCA and YEDC plates were incubated at 30 °C for 48 h and *Bacillus cereus* Agar Base plates at 37 °C for 48 h, in aerobic conditions. After incubation, the grown colonies were counted, and data were expressed as colony-forming units per gram (CFU/g) of risotto.

2.3. Nutritional analysis

2.3.1. Proximal analysis

Proximal analysis of recipes A and B was done by an accredited external laboratory (Eurofins Chemical Control, Cuneo, Italy). Moisture, ash, and total fats were measured using standard methods (Istituto Superiore di Sanità, 1996). Protein analysis was conducted following the Dumas method using a flash EA 1112 NC analyzer (Thermo Fisher Scientific Inc., Waltman, USA). Total dietary fiber was determined using the AOAC method 2009.01. Fatty acids (% methyl esters) and sugar profiles were determined using a GC-FID method and an HPAEC-PAD method, respectively. A mass spectrometry method based on inductively coupled plasma was used for determining sodium, phosphorus, and copper content after pressure digestion (EN 16943, 2017). Carbohydrate content was calculated as a difference. Saturates, monounsaturated and polyunsaturated fatty acids, and trans fatty acids were calculated from the fatty acid analysis. Salt was calculated from sodium

Table 1
Formulations of recipe A and recipe B.

	Recipe A	Recipe B
Ingredients	**Weight (g)**	**Weight (g)**
Wholegrain rice	85	85
Mushrooms (*Agaricus bisporus*)	70	0
Chickpeas	70	0
Lentils	0	70
Borlotti beans	0	70
Onion	0	30
Rosemary	3	1
Mushroom powder (*Boletus edulis*)	2	0
Garlic	1	0
Sage	0	1
Extra virgin olive oil	12	14
Total	243	271
Water	60	65

content according to Regulation (EU) No 1169/2011. The energy value was calculated using the energy factors provided in Regulation (EU) No 1169/2011.

2.3.2. Starch in vitro digestibility

Starch *in vitro* digestibility of pouches was measured using Englyst's procedure (Englyst et al., 1999). A hundred and fifty g of pouches content was transferred into a closed container and microwave heated at 800 W for 3 min, minced with a meat mincer (Adler 4808), and passed through a 7 mm hole plate to simulate the oral phase. Two g of the minced sample were added with 10 mL of pepsin (P7000 ≥ 250 U/mg solid, Sigma, Milan, Italy)-guar (G4129, Sigma, Milan, Italy) solution (0.05 M HCl) and incubated at 37 °C for 30 min in a 3 Hz shaking water bath (Dubnoff, Julabo, Turin, Italy) to simulate the gastric phase. Then, 10 mL of sodium-acetate solution 0.25 M, 5 mL of the enzymatic mix containing pancreatin (P7545 8xUSP, Sigma, Milan, Italy), amyloglucosidase (A7095 ≥ 260 U/mL aqueous solution, Sigma, Milan, Italy), and invertase (I4504 ≥ 300 units/mg solid, Sigma, Milan, Italy), and 5 glass balls (Ø 1.5 cm) were added, and the suspensions were incubated at 37 °C for 2 h in a 3 Hz shaking water bath to simulate intestinal phase. At 20 and 120 min of the intestinal phase, 1 mL aliquot was taken to determine the concentrations of glucose at these times (G_{20} and G_{120}, respectively). G_{20} and G_{120} were determined using a bioanalytical glucose analyzer with a combined enzymatic-electrochemical system (YSI 2300 Stat Plus, Yellow Spring Instruments, OH, USA). The presence of free sugar glucose (FSG) in the samples was also determined. An amount (0.5 g) of undigested risotto was added with 5 mL of distilled water, vortexed for 2 min, and centrifuged (PK110, ALC; Newport Pagnell, England) for 15 min at 1972 × *g*. Then, 1 mL of supernatant was collected and analyzed with the glucose analyzer. The following data were obtained: rapidly available glucose (RAG), which is the amount of glucose released during the first 20 min (G_{20}); slowly available glucose ($SAG = G_{120} - G_{20}$), which is the glucose released between G_{20} and G_{120} min of intestinal digestion. These parameters and the conversion factor to convert the glucose concentration in starch amount (0.9) were used to obtain rapidly digestible starch ($RDS = (G_{20} - FSG) \times 0.9$) and slowly digestible starch ($SDS = (G_{120} - G_{20}) \times 0.9$).

Resistant starch (RS) and total starch of microwave-heated samples were determined using the Resistant Starch Assay kit (Megazyme International, Wicklow, Ireland) (McCleary & Monaghan, 2002). The units of RDS, SDS, and RS were expressed in grams of starch per 100 g of total starch (% of total starch on a wet basis).

2.3.3. Glycemic index

The evaluation of GI of RTE meals was carried out in agreement with the guidelines defined in ISO 26642:2010. According to the Helsinki declaration on human rights, all the subjects gave written informed consent before the study started. Subjects were enrolled after a screening for the general status and the exclusion criteria were: age < 18, BMI > 30 kg/m^2, celiac disease, metabolic disorders (e.g., diabetes, hypertension, dyslipidemia, glucose intolerance), chronic use of medications for any conditions (including psychiatric diseases), use of dietary supplements affecting metabolism, and anemia. Test (RTE, recipes A and B) and reference (glucose solution on 2 occasions) meals were randomly administered to the subjects for a total of 4 different occasions with a minimum washout of 1 day. The subjects were asked not to consume food or beverages (except water) 12 h before the test. The day before each test, participants were instructed to avoid foods that might interfere with glucose metabolism the next day, based on the indication of the ISO 26642:2010, and other foods rich in indigestible carbohydrates to avoid the second meal effect. On the day of the test, fasted volunteers were asked to eat a portion of either RTE or a reference meal containing 50 g of available carbohydrates. The RTE meal was warmed in the microwave (800 W) for 3 min before serving. The subjects consumed the meal, served with 500 mL of water, over a 12–15 min period. The capillary whole blood was obtained by a finger prick (Accu-Chek Advantage System, Roche Diagnostics Limited, Lewes, UK) before the meal (fasting) and at 15, 30, 45, 60, 90, and 120 min after the meal consumption. Blood samples were collected in heparinized tubes and glycemia was determined using a bioanalytical glucose analyzer (YSI 2300 Stat Plus, Yellow Spring Instruments, OH; USA). The GIs were calculated from 120 min incremental postprandial blood glucose areas under the curves, obtained by plotting blood glucose concentration against time, using 50 g of available glucose as the reference food.

2.4. Risotto ingredients texture

The texture of rice, chickpeas, and lentils was measured at 0, 3, and 12 months of shelf life using a texture analyzer (Stable Micro Systems, Godalming, UK) equipped with a 25 kg load cell aluminum cylindrical probe with a diameter of 40 mm as previously described (Federici et al., 2021) with some modifications. Three grams of rice kernels, chickpeas, and lentils were spread in a single layer on the base instrument and compressed at a speed of 0.1 mm/s to 75% total strain. Hardness before and after microwave heating (800 W for 3 min) was taken as the force (N) at maximum compression using Texture Expert software (Stable Micro Systems Godalming, UK). At least 20 kernels of rice, 10 chickpeas, and 10 lentils were measured for every pouch and at least 3 pouches were analyzed for every shelf life point.

2.5. Risotto acceptability by consumers

Sensory analysis of RTE meals was carried out in separate sessions for recipes A and B for the fresh product and after 3 and 12 months of storage at room temperature. Fifty untrained voluntary adult consumers were recruited. Participants gave informed consent via the statement "I am aware that my responses are confidential, and I agree to participate in this survey" where an affirmative reply was required to enter the sensory study. They were able to withdraw from the study at any time without giving a reason.

For each test, a 15 g sample portion (designated with three-digit random number codes) was presented in a balanced randomized order. Each portion was placed on a white plastic dish, covered with another dish to prevent drying, and then heated in a microwave (800 W) for 3 min before serving. The serving temperature was approximately 60 °C. Judges were provided water to rinse their palate upon need.

Judges were asked to fill out a questionnaire that comprised 3 sessions:

1) Information about sex and age (Which of the following ranges does your age fall into? 18–25, 26–35, 36–45, 46–55); habits in the consumption of RTE meals (How often do you eat ready meals? Never, once a month, once a week, 2–4 times a week, or more), and their type of diet (Are you following a specific diet? None, losing weight, vegan, vegetarian, lactose- or gluten-free, other).
2) Product sensory evaluation through acceptability and Check All That Apply (CATA) tests.
i) The acceptability test asked to rate the samples from 1 to 9 (1 = dislike extremely, 2 = dislike very much, 3 = dislike, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like, 8 = like very much, and 9 = like extremely) for the following attributes: appearance, texture, aroma, flavor, and overall acceptability.
ii) CATA test asked to taste the rice samples and checked the attributes that were believed to apply to the product selecting them to the following list: good appearance, bad appearance, healthy, hard, al dente, well-cooked, gummy, soft, sticky, savory, tasteless, good flavor, bad favor, bad, mediocre, good, excellent. Data were collected as the times each attribute was selected for each sample and then converted to a percentage of the total consumer number. Attributes in the CATA test questionnaire were presented in a randomized order.

3) Information about willingness to buy the RTE meal (After tasting the product, would you be willing to buy it? Absolutely not, probably not, no/yes, probably yes, absolutely yes).

2.6. Statistical analysis

For every *in vitro* analysis, at least three replicates were performed and for each of these, three technical repetitions were done. The results of starch *in vitro* digestibility and texture analysis were expressed as mean ± standard deviation (SD); the results of the GI test were expressed as mean ± standard error of mean (SEM). The normality of the data was verified through the Shapiro-Wilk test ($p < 0.05$). To verify the homogeneity of variance between means, the Levene test ($p < 0.05$) was carried out. For the normally distributed data, the means obtained from replicate tests were subjected to the one-way analysis of variance (ANOVA) ($p < 0.05$). Differences between the means were assessed using Tukey's post-hoc test. The non-normal distributions (texture data in the specific case) were analyzed using the Kruskal-Wallis test (non-parametric one-way ANOVA, $p < 0.05$). Differences between the means were assessed with pairwise comparisons. All the statistical tests were carried out using SPSS Statistic software (Statistics for Data Analysis version 28, SPSS Inc., Chicago, IL).

3. Results and discussion

Nowadays, the convenient nature of RTE meals makes them very popular with consumers. In 2019, RTE represented a global revenue of 98.12 billion USD. The European market had the major share with revenue equal to 29.83 billion USD. In 2024, the global revenue is expected to reach 122.95 billion USD and 39.36 billion USD in Europe (Mordor Intelligence Analysis, 2018). However, despite the popularity of these products, their nutritional quality is generally low, as recently demonstrated in a survey of 177 RTE meals sold in Hungary (AlOudat et al., 2021). Therefore, in the present study, two RTE meals containing primarily BR and pulses (recipe A = BR, chickpeas, and mushroom; recipe B = BR, lentils, and beans) were developed (Table 1) with the aim of having nutrient-dense products rich in healthy (e.g. copper) and low in unhealthy (e.g. sodium) nutrients and with a low GI. Onion, garlic, sage, rosemary, and EVO oil were also added to the formulations making them complete meals ready to eat only after 3 min of microwave heating. Table 2 reports the nutritional declaration according to Regulation (EU) No 1169/2011. The energy per 100 g was 182 kcal (766 kJ) for recipe A and 167 kcal (703 kJ) for recipe B, which corresponds to about 20% of the daily energy intake for an average adult considering the entire serving in a pouch (250 g). According to Regulation (EU) No 1924/2006, both RTE formulations can bear several nutrition claims. Recipes A and B are "sources of fiber" since they contain > 3 g of fiber per 100 g, and "sources of phosphorus and copper" since they provide > 15% of the nutrient reference values per 100 g. Both recipes could also be claimed as "low-saturated fat" having a sum of saturated fatty and *trans*-fatty acids lower than 1.5 g per 100 g which provide < 10% of energy. Finally, recipe B is a "low sodium/salt" product since it had no > 0.12 g of sodium per 100 g. The higher salt content of recipe A than recipe B was probably due to the presence of mushroom powder. The formulation might be revised to lower the salt/sodium content by at least reaching 0.23 g of sodium, which is the benchmark value recently suggested by the World Health Organization (2021) for "Pasta, noodles, and rice or grains with sauce or seasoned" subcategory of "Ready-made and convenience foods and composite dishes" category. Therefore, it is possible to state that the two developed risotto formulations are nutrient-dense meals and have better nutritional content than the average nutritional profile of 177 traditional and innovative RTE Hungarian meals recently collected (AlOudat et al., 2021). Indeed, the two recipes had lower content of fat, saturated fatty acids, sugar, protein, and sodium and higher values of carbohydrates than the median values of the investigated RTE meals (AlOudat et al., 2021).

Table 2
Nutrition declaration of recipes A and B.

	Recipe A		Recipe B	
	per 100 g	% RI per 250 g*	per 100 g	% RI per 250 g*
Energy value	766	23	703	20
kJ	182		167	
kcal				
Total fat, g	6.05	23	3.94	15
Saturated fat, g	0.95	13	0.69	8
Monosaturated fat, g	2.97	–	2.23	–
Polyunsaturated fat, g	2.13	–	1.03	–
Carbohydrates, g	25.32	25	23.93	23
Sugars, g	0.33	1	0.65	2
Dietary fiber, g	3.64	–	5.77	–
Protein, g	4.86	25	6.13	30
Salt, g	0.67	28	0.22	10
Phosphorus, mg	138	50	143	50
Copper, mg	0.21	53	0.21	53

*RI = reference intake for adults (8400 kJ/ 2000 kcal), according to Regulation (EU) n. 1169/2011. The value of 250 g refers to one serving.

The RTE risotto pouches were produced by thermal sterilization which is the commercial treatment most commonly used to make an RTE meal safe and shelf-stable at room temperature for at least 12 months (Yu et al., 2017). The effective sterilization procedure and the maintenance of the microbiological safety of the pouches were verified immediately after the production and after 3 and 12 months of storage with the analysis of total mesophilic and spore-forming bacteria, yeasts and molds, and *B. cereus*. Results showed no microbial growth over 12 months with a microbial count lower than the detection limit (100 CFU/g) for all the microbial species investigated, confirming a correct sterilization cycle and the efficacy of the thermal treatment.

3.1. In vitro starch digestibility

The *in vitro* starch digestibility of recipes A and B was investigated at 0, 3, and 12 months after microwave heating to determine the values of RDS, SDS, and RS. RDS is the amount of starch digested in the first 20 min and can be useful for predicting glycemic responses (Boukid et al., 2019). The results are shown in Fig. 1. The amount of the different starch fractions was related to the starch present in all the ingredients used for the preparation of the pouches, in particular BR, chickpeas, lentils, and beans. RDS was the most abundant starch fraction, while SDS and RS represented smaller fractions in both recipes. The RDS fraction did not change over time and was always higher ($p < 0.05$) in recipe B than in recipe A (60.33 ± 2.92% and 45.85 ± 2.46% as the average value of the three times investigated, respectively). The fraction of SDS was unchanged ($p > 0.05$) during the 12-month storage period in both recipes and was always higher in recipe A than in recipe B (25.97 ± 1.04% and 19.16 ± 0.19% as the average value of the three times investigated, respectively). This finding could be due to the different digestibility of the ingredients present in the formulations. Both recipes contained BR, whose low digestibility is probably attributed to the resistance of its bran layer to water penetration into kernels during digestion. In addition, in the BR bran layer, phenolic compounds, such as phytic acid, are present which may contribute to the partial inhibition of digestive enzymes decreasing digestibility (Mohan et al., 2017). However, recipe A contained chickpeas that include starch known as less digestible than the starch in lentils and beans (Brighenti et al., 1995). Also, the small size of lentils might have favored more extensive cooking, water penetration, and starch gelatinization as compared to beans and chickpeas (Singh et al., 2021).

The fraction of RS did not change in recipe B, while increased ($p < 0.05$) after 3 months in recipe A, and then it maintained constant for up to 12 months. Moreover, comparing the two recipes, the amount of RS was similar at month 0 but different at 3 and 12 months, with the highest

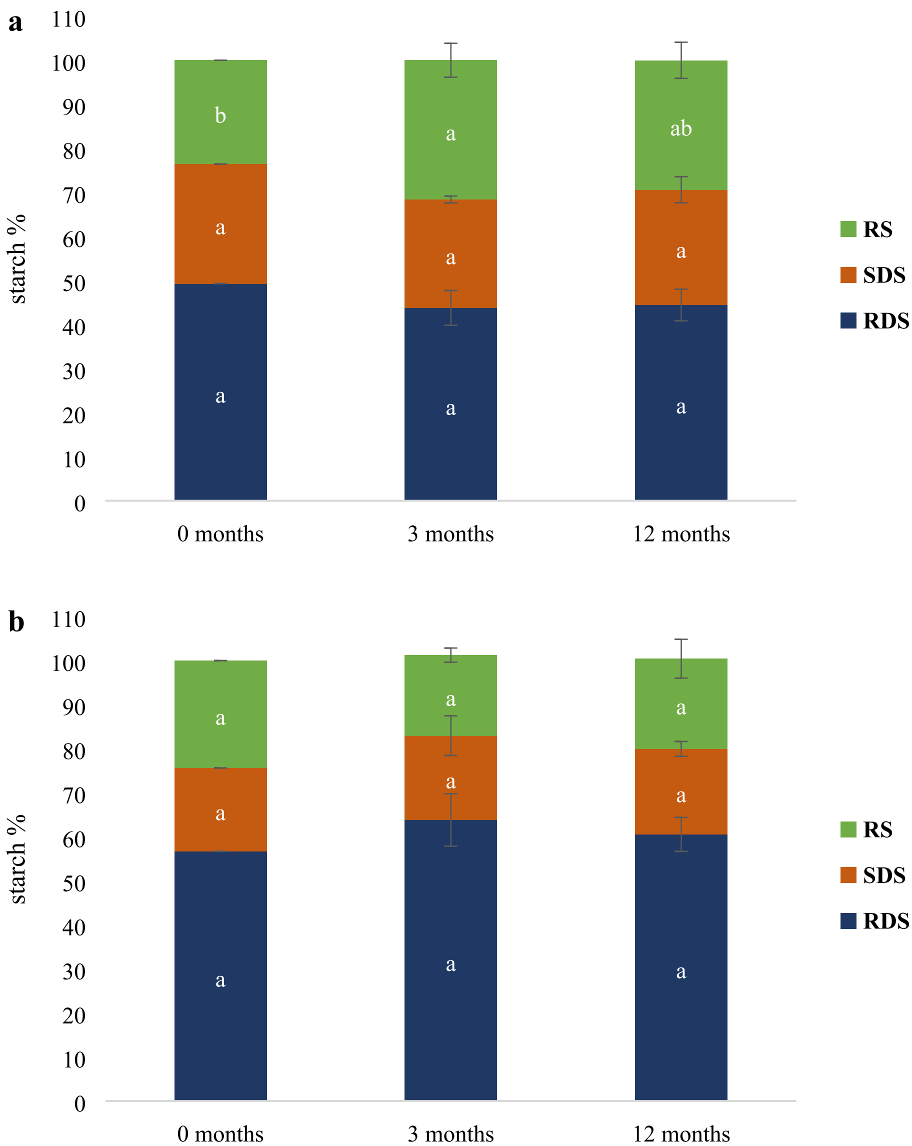

Fig. 1. Starch *in vitro* digestibility (mean ± SD) of recipe A (a) and recipe B (b) during shelf life. Each starch fraction is expressed as g/100 g of total starch. For each recipe and then starch fraction, different letters represent values significantly different ($p < 0.05$). Rapidly digestible starch (RDS); slowly digestible starch (SDS); resistant starch (RS).

($p < 0.05$) values in recipe A, suggesting that this fraction was affected by the different types and amounts of pulses. The RS is the starch that is not digested in 120 min since it can be entrapped in intact cells (RS1), it could have a molecular structure that hinders the enzymatic activity (RS2) and it could be related to retrograded starch whose amylose and amylopectin are re-polymerized during cooling in a more thermodynamic ordered state (RS3) (Chang et al., 2021). The latter process might have occurred in all the risotto ingredients containing starch, also in agreement with the results of the texture analysis (Table 3) which showed an increase in the hardness during shelf life. Differently from BR and lentils, the chickpeas' morphology and cell-wall structure represent an important barrier to the permeability of enzymes and water (Xiong et al., 2019) and the retrograded starch was likely not re-gelatinized during microwave heating (Carreira et al., 2004). As reviewed by (Fuentes-Zaragoza et al., 2010), the presence of a certain amount of RS in meals has health benefits, along with the lowering of GI.

Pulses are key ingredients in healthy meals since they are a plant-based protein source that nowadays is preferred due to their low environmental impact, animal welfare, and religious and cultural reasons (Keskin et al., 2022). Moreover, the incorporation of BR and pulses in

Table 3
Hardness before and after microwave heating (mean ± SD) of rice, chickpeas, and lentils at 0, 3, and 12 months of shelf life. Different letters within the column of the same ingredient mean values significantly different ($p < 0.05$). The asterisk means values significantly different ($p < 0.05$) between the hardness before and after the microwave heating at the same time.

		Months	Hardness (N)	Hardness MW§ (N)
Recipe A	**Rice**	0	12.84 ± 6.31^{b}	5.20 ± 2.35 ^{ab*}
		3	18.46 ± 9.88 ^{a}	6.11 ± 4.10 ^{a*}
		12	18.52 ± 8.52 ^{a}	4.90 ± 2.19^{b*}
	Chickpeas	0	4.67 ± 1.12^{b}	3.51 ± 0.93^{b*}
		3	4.40 ± 1.55^{b}	4.33 ± 1.24 ^{a}
		12	5.24 ± 1.03 ^{a}	4.54 ± 1.17 ^{a*}
Recipe B	**Rice**	0	16.60 ± 8.18^{b}	6.47 ± 2.53^{b*}
		3	16.31 ± 8.20^{b}	5.53 ± 2.12^{b*}
		12	27.61 ± 15.27 ^{a}	9.52 ± 5.47 ^{a*}
	Lentils	0	3.17 ± 0.85^{b}	2.79 ± 1.14^{b}
		3	3.29 ± 1.01^{b}	2.62 ± 0.90^{b*}
		12	4.08 ± 1.23 ^{a}	3.51 ± 1.55 ^{a*}

§ MW = microwave heating (800 W for 3 min).

the diet contributes to slowing the gastric emptying rate resulting in an earlier sense of fullness during a meal, reduced hunger, and increased satiety (Pletsch & Hamaker, 2018; Singh & Pratap, 2016).

3.2. Glycemic index

The results obtained *in vitro* suggest that the two RTE meals containing BR and pulses might have a low GI. This was confirmed by the results obtained from the *in vivo* study. A group of twelve healthy adult volunteers (all females; 29.1 ± 10.9 years old; mean ± SEM), with a body mass index of 21.1 ± 2.0 kg/m^2 (mean ± SEM) participated in the *in vivo* study. The glycemic response curves following the consumption of the two risotto meals were lower than that of the reference food (i.e., glucose) (Fig. 2). Incremental glycemic curves were used to calculate the GI values that were 43.5 ± 6.8 and 31.8 ± 6.5 for recipes A and B, respectively. Both formulations are low GI meals as the GI values were lower than 55 (Atkinson et al., 2008). The GI of the formulations was determined immediately after the production of the pouches and not after 12-month storage since no changes in GI were assumed because of the results of the *in vitro* starch digestibility. GI is linked with the amount of RAG, which in turn is directly correlated to the amount of RDS (Englyst et al., 1999) that remained unchanged during storage.

The choice of including BR instead of WR probably had a key role in lowering the GI of formulations (Malik et al., 2019; Yu et al., 2022) since boiled BR has a GI value of 68 ± 4, which is lower than that of boiled WR (73 ± 4) (Atkinson et al., 2008). Moreover, the presence of pulses in the two formulations likely contributed to reducing the GI since chickpeas, beans, and lentils have low GI (28 ± 9, 29 ± 9, and 32 ± 5, respectively) (Atkinson et al., 2008; Foster-Powell et al., 2002). This result is consistent with the previous study of Mohan et al. (2014) who found that the addition of legumes to BR further decreased its glycemic response. It is also worth highlighting that the added amount of oil was low in both recipes (12–14 g per 100 g), as well as the total fat content. The low GI of the developed meals was not related to their fat content, which slows down gastric emptying and delays the rise in blood glucose concentration (Gentilcore et al., 2006).

3.3. Risotto texture

Food texture is one of the main sensory attributes judged by consumers and it plays a crucial role in influencing consumers' liking and preference for a food product (Tunick, 2011). In complex foods such as RTE meals, the perceived textural sensation is a result of the combination of textural attributes of the single ingredients. The hardness of the main ingredients of recipes A and B was, therefore, separately investigated. In particular, BR kernels, chickpeas, and lentils were analyzed after opening the pouches and after microwave heating to replicate usage conditions at the selected three time points (0, 3, and 12 months). Mushrooms and beans were not analyzed since they lost their structural integrity and were already mashed after the preparation of the pouches (0 months). Texture analysis results are summarized in Table 3. Hardness increased during storage for all the analyzed ingredients both before and after microwave heating, even though this process partially softened the products by a partial gelatinization of retrograded starch. In recipe A, BR hardness before microwave heating increased significantly ($p < 0.05$) after 3-month storage, then remained constant until 12-month storage. After microwave heating, rice hardness at the end of storage was similar to the value measured immediately after the preparation of the pouches but different ($p < 0.05$) comparing it with the hardness at 3 months. Chickpeas hardness (recipe A) before microwave heating was significantly ($p < 0.05$) higher at 12 months than at earlier time points, and, after microwave heating, the increase was significant already after 3-month storage. In recipe B, the hardness of BR and lentils had a similar trend being significantly higher ($p < 0.05$) at 12-month storage both before and after microwave heating than at other time points. The hardness increase of the ingredients during the storage is likely related to the retrogradation of starch, a process that leads to the structural rearrangement of starch molecules with the formation of crystalline–paracrystalline structures (Chang et al., 2021). Also, hardness was influenced by microwave heating: comparing the values of the hardness of BR, chickpeas, and lentils at the same storage time before and after the microwave heating, the values after the heating were always significantly lower ($p < 0.05$), with the only exception of chickpeas at 3 months.

3.4. Consumers acceptability

Consumers' acceptability represents a crucial aspect in the development of an RTE meal. Therefore, we recruited a heterogeneous group of possible buyers of RTE products to evaluate the acceptability of developed meals during their shelf life. Fifty-two percent were female and the age ranged between 18 and 55 years old. Thirty-seven percent of

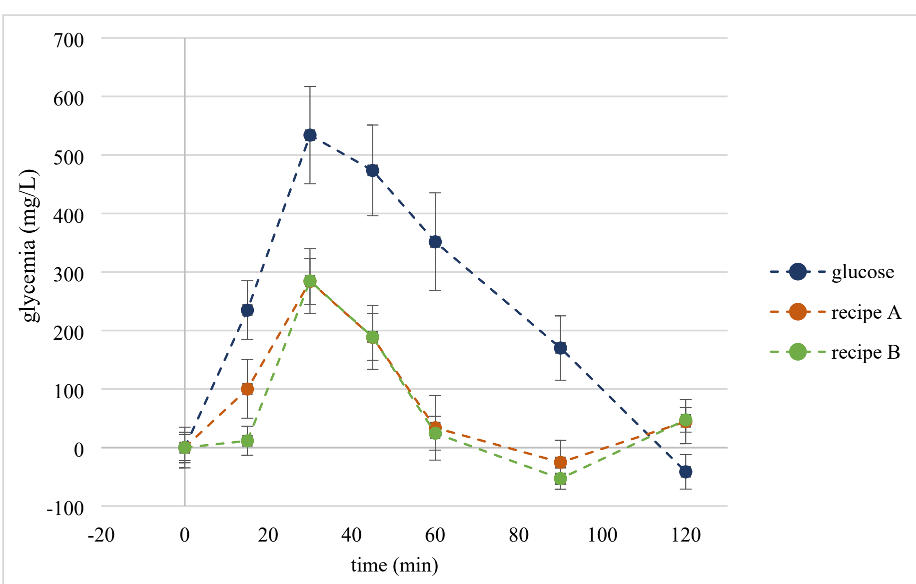

Fig. 2. Incremental blood glucose postprandial curves of recipe A, recipe B, and glucose. Values are expressed as mean ± SEM (n = 12).

the participants do not consume RTE meals while 63% ate at least once a month, confirming that nowadays the consumption of RTE meals concerns most people (Chaffee et al., 2022; Choi, 2022). Regarding the type of diet, 79% of participants followed a normal diet, and the remaining followed a specific diet (losing weight, vegetarian/vegan, lactose/gluten-free, and others). In this regard, both formulations are gluten- and lactose-free, and vegan.

Sensory analysis results are reported in Fig. 3 (acceptability test) and Fig. 4 (CATA test). The spider plots (Fig. 3) show how the appearance, texture, flavor, aroma, and overall acceptability of recipe A and recipe B changed during the shelf life, while the histograms report how the frequency of the attributes used to describe the meals varied between 0 and 12 months. For both formulations, after 12-month storage, appearance, texture, flavor, aroma, and overall acceptability slightly decreased without significant ($p > 0.05$) differences and remained in the range of 6 – 7 for recipe A and 5.5 – 6.5 for recipe B. Recipe A obtained higher scores than recipe B alluding that chickpeas and mushrooms were more appreciated by the consumers than the lentil and bean formulation. This finding was confirmed by the CATA test (Fig. 4) in which recipe A was judged more “savory” and less “tasteless” than recipe B, probably for the different salt content, and, in turn, recipe B was considered more “mediocre”. The most selected descriptors for both recipes were “good”, “good flavor”, “gummy”, “well cooked”, “al dente”, “healthy”, and “good appearance”. This suggests that among all the proposed descriptors, these were the most important for the consumers to judge these two meals. After 12-month storage, both meals were considered more “mediocre” and less “good”, and “healthy”, even if the last one is a consumer perception and not a tangible descriptor, and having “good flavor”. However, these attributes did not significantly change ($p > 0.05$) over time, suggesting that consumers might perceive no alterations to the main sensory attributes of the product during storage nor the development of off-flavors which usually occur in BR meals due to the oxidation of lipids in the outer bran layer and potentially influence consumer perception and acceptance (Gondal et al., 2021).

The higher acceptability of recipe A than recipe B was confirmed by the questionnaire on the willingness of purchasing: 48% of the participants would probably buy the meal with BR and chickpeas compared to 33% for recipe B while 37% would not probably buy formulation with

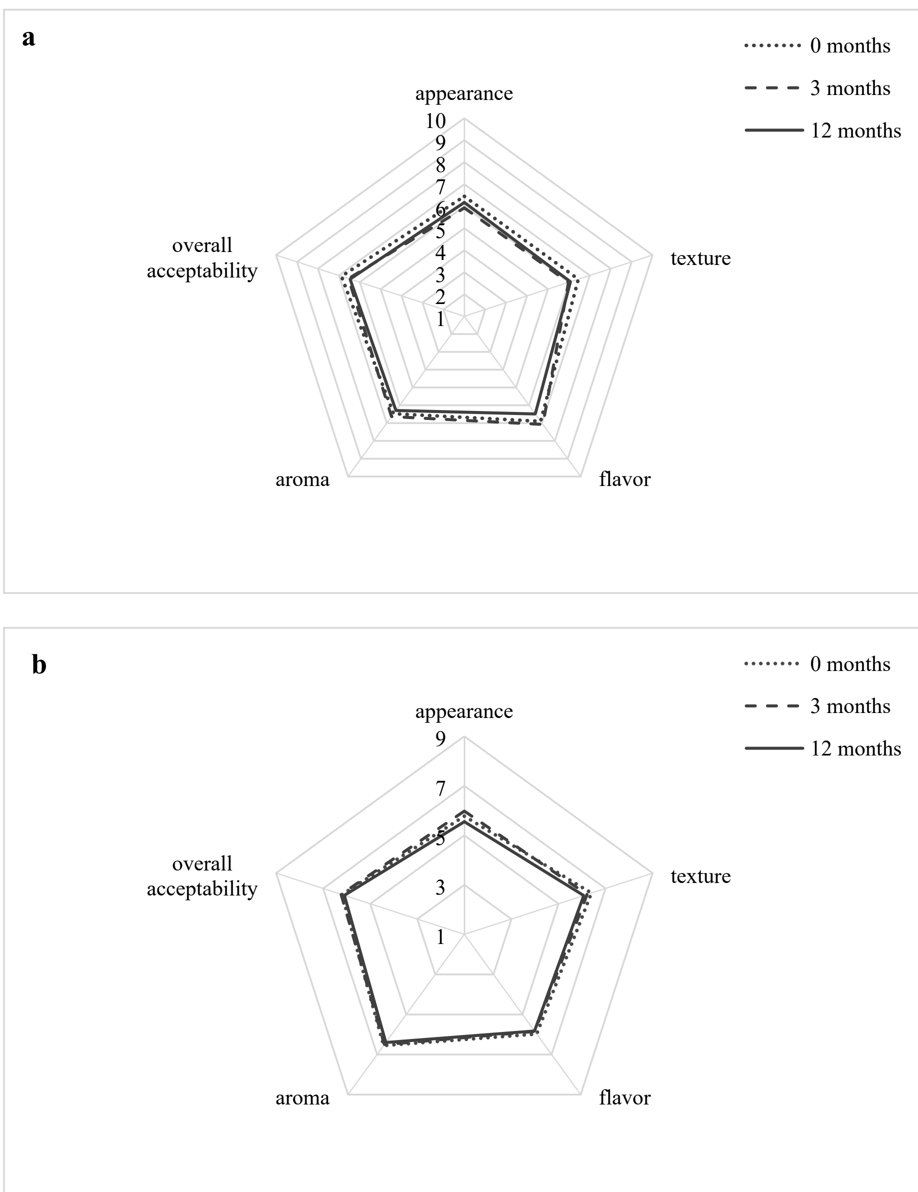

Fig. 3. Results of acceptability test during the shelf life of recipe A (a) and recipe B (b).

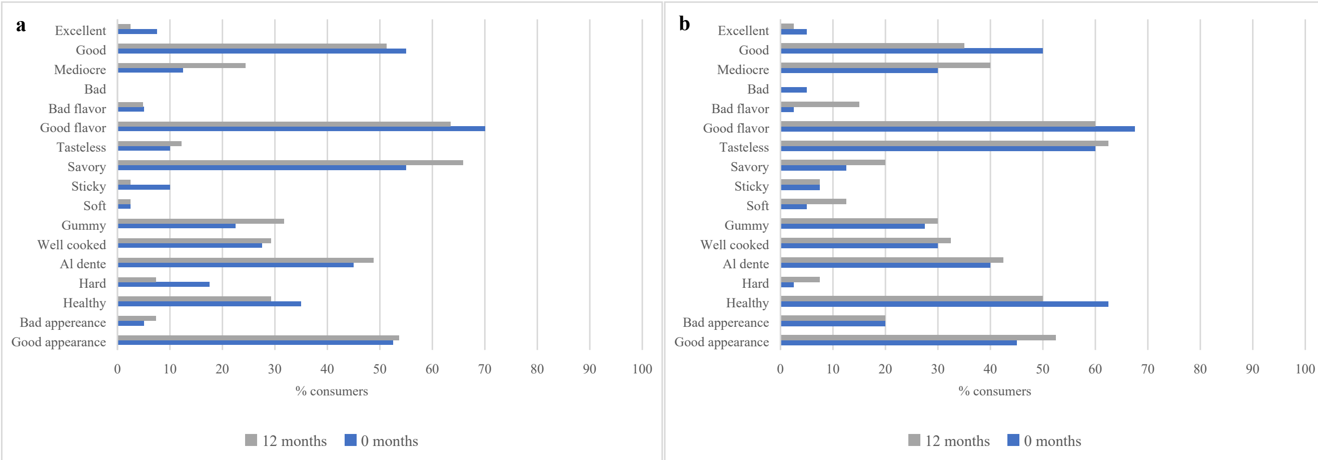

Fig. 4. Results of check all that apply test of recipe A (a) and recipe B (b) during shelf-life.

lentils and beans versus the 21% for recipe A. The evaluation of the sensory profile of the meals is an important driver of consumer acceptance and the sensory attributes have a strong influence on product selection, consumption, and purchase decisions.

3.5. Conclusion

This study demonstrates how nutrient-dense and healthy shelf-stable RTE meals can be developed and well-accepted by a heterogeneous group of consumers. The risotto formulations, which contained BR and pulses, i.e. chickpeas, lentils, and beans, can bear several nutrition claims such as a source of fiber, phosphorus, copper, low-saturated fat, and low sodium/salt content. Moreover, both RTE meals were proven to have a low glycemic index. At the end of the storage, participants could not detect the increase of the hardness, despite texture analysis on single ingredients proving the contrary, suggesting that the developed RTE meals are stable over time from a sensory as well as a microbiological point of view. This study fills a gap related to the evaluation of the shelf life of sterilized brown rice in terms of changes in starch digestibility and texture, which are topics barely explored. Moreover, it presents promising results in the development of low glycemic RTE meals containing brown rice and pulses and could help to foster the consumption of brown rice, which is still low.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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