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Application of fermented black rice along with malt coating containing potential probiotic yeast to produce a functional cupcake

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ABSTRACT

Production of probiotic (PRO) cereal-based products is an important strategy to increase consumption amounts of these beneficial microorganisms in our daily diet worldwide. In the present study, textural features, sensory properties and viability of an adjunct PRO culture were determined in supplemented cupcake with fermented black rice (FBR) and coated with malt. Based on the obtained results, survival of the PRO yeast in malt edible coating reached to 10^6 CFU/g after 6 days of storage in cupcake samples supplemented with FBR. In addition, crumb hardness (1529.77 g) and gumminess (962.14) of the produced cupcake supplemented with FBR were significantly (P<0.05) higher than those of the control sample. Meanwhile, proper porosity and overall acceptability were observed for the aforementioned FBR added cupcake. Accordingly, application of a potential PRO yeast via malt coating as a proper carrier can maintain viability of this adjunct culture during shelf-life period of the product in the recommended dose for PRO products. Considering the health-promoting potentials of PRO and consumed amounts of cupcake, it is a good choice to consume a high dose of PRO in our today's diet style.

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1. Introduction

Considering limitations associated with dairy-based probiotic (PRO) products, development of novel categories of PRO foods like cereal-based PRO is an important issue in this field. Moreover, application of PRO yeasts (PY)s in food industries has received more attention in recent years. Considering non-transferable antibiotic resistance of PYs, their promising potential to tolerate stressful conditions of the gastrointestinal tract (GIT) and some food processing conditions, these eukaryotic cells can be proper candidates instead of PRO lactic acid bacteria (LAB) in food sector [1].

Today, the use of pseudo-cereals in production of functional foods is increasing due to their suitability for growth of PRO cultures. In addition, these functional substrates contain dietary fibers and prebiotic (PRE) compounds like non-digestible carbohydrates. Combined application of PRO and these PRE improves quality characteristics of the bakery products such as their textural features and sensory properties. Black rice or forbidden rice is a type of pseudo-cereals that is cultivated in East Asian countries. This rice is also rich in antioxidant compounds and contains free fatty acids, triglycerides and phospholipids as superior substrate to produce functional foods [2, 3]. Sourdough (SD) as a stressful ecosystem is a fermented flour-water mixture that is proper substrate to isolate potential PRO, as well as to enhance technological functionalities of the bakery products [4]. PRO properties of some indigenous LAB isolated from different SDs like amaranth [5], sprouted clover seeds [6], acorn [7], quinoa [8], oat [9], rice bran [10, 11], whole barley [12] and whole wheat [13], as well as some SD yeasts isolated from buckwheat SD [14, 15] and wheat germ SD [16] have been verified. Moreover, emerging techno-functional capabilities of SD yeasts as potential starter cultures in baked goods have also been reported [17].

PRO cake is one of the most important confectionary products that received considerable attention due to the health-promoting potentials of these beneficial microorganisms. Although there are some reports about production of PRO cakes containing PRO LAB [18], while PYs have been rarely studied yet as adjunct cultures in PRO cakes [19]. The use of edible coatings is also one of the simplest and effective approaches to produce PRO cereal-based products [20]. In this regard, Arslan-Tontul et al. (2019) produced a cake covered with chocolate containing PRO *Saccharomyces boulardii*. According to the results, the coating layer protects PRO and affect quality characteristics of the product [21].

The aims of the present study were to evaluate the effects of fermented and non-fermented black rice (NFBR) with and without malt coating containing a PY strain on the survivability of the PRO during storage, as well as on textural features and sensory attributes of the produced cupcakes.

2. Materials and Methods

Raw materials

Proximate composition of wheat and black rice flours were determined according to the reference protocols [22]. Accordingly, white wheat flour (68% extraction rate) contained 13.10% moisture, 11.25% protein (N × 5.70), 0.60 fat, 74.50 total carbohydrate and 0.55% ash. Black rice flour had also 7.83% protein (N × 5.70), 1.09% fat, 12.65% moisture, 0.51% ash and 77.92% total carbohydrate. The yeast used in the present study (*Rhodotorula mucilaginosa*) was isolated from fermented black rice (FBR). Furthermore, its PRO properties were characterized, and it was molecularly identified in our previous study [23]. Chemical reagents and microbial culture media were also purchased with analytical grade.

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Cupcake production process

To produce the cake batter, a mixture of vegetable oil (14.25 g) and sugar (18 g) was prepared using a kitchen mixer (Sapor, China) at medium speed for 5 min until it becomes creamy. Then, the whole egg (18 g) was added into the mixture and stirred at high speed for 5 min. Subsequently, wheat flour (25 g), baking powder (0.5 g) and water (8 mL) were mixed at low speed for extra 1 min. Finally, the dough was baked in an electric oven (Leto, UK) at 175 °C for 30 min [24]. Supplemented cakes were also produced with mixture of spontaneous FBR (fermented at 28 °C for 24 h with pH = 5.5) or NFBR and wheat flour with and without malt coating containing the PY with 10^8 colony forming units (CFU)/g of the sample. Proper amount of FBR and NFBR was also determined as 10% w/w according to the pretreatments. It should be noted that all the samples had the same amounts of water (wet basis) content and dry basis weight.

Assessment of PY survival in the produced cakes

To evaluate the survivability of the PY isolate in the produced cupcake samples on the second, fourth and sixth days after baking (shelf-life period of the cupcake), serially ten-fold diluted samples were prepared in sterile ringer solution, and then they were surface plated on yeast extract glucose chloramphenicol (YGC) agar medium. Subsequently, the number of live yeasts was determined after 48 h of incubation at 25 °C compared to the initial population (10^8 CFU/g).

Textural features of the produced cakes

To determine textural characteristics of the produced cupcakes including their crumb hardness and gumminess, texture profile analysis was used 2 h after baking. For this purpose, the compression test was done using a texture analyzer (Stable Micro System, UK) with a cylindrical probe with a diameter of 10 mm and a speed of 3 mm/s. After applying the necessary force to create 30% compression in the initial thickness, the mentioned characteristics were measured through drawing the force-distance curve [21]. Water activity of the produced samples (*aw*) was also determined using an *aw* meter (Novasina-LabSwift, Switzerland).

Sensory properties of the products

Sensory characteristics of the produced cupcakes were evaluated in a time interval of 2 h after baking through a panel test. Trained judges investigated the crust color, taste, shape and odor of the produced cakes based on a five-point hedonic scale (1 the lowest and 5 the highest), and finally overall acceptability (OA) was calculated as the mean of the aforementioned properties [21].

Statistical analysis

The results of the present research were statistically analyzed based on a completely randomized design and one way analysis of variance (ANOVA) with three replicates using SPSS software (version 20). Means were compared using the least significant difference (LSD) test and the paired sample *t*-test at $P<0.05$. The independent-samples *t*-test was employed to determine significance of differences between two different samples. Microsoft office Excel 2016 was also used to draw the charts.

3. Results and Discussion

Survivability of the probiotic yeast in the produced cakes

The viability of PRO *R. mucilaginosa* in cupcake samples with malt coating was constant (equal to 10^6 CFU/g) during storage period (on the second, fourth and sixth days after baking) at 25 °C for 6 days, and population reduction by two logarithmic cycles was observed compared to the initial population.

In the same vein, application of germinated black glutinous rice in processing of Khao-Maak as a fermented rice cake in Thailand resulted in more than 97% survival rate of PY *S. boulardii* after *in vitro*

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gastrointestinal tests due to PRE potentials of the used substrate in the study of Cheirsilp et al. [19]. The same results about protective effect of inulin as a commercial PRE were also reported in the study of Banerjee et al. [25]. The viable counts of added PRO in the functional cream-stuffed cake based on rice and sweet potatoes were also higher than the recommended dose for health benefits in the study of Ramadan et al. [26]. PY have several potential techno-functional capabilities in food industries as adjunct, starter or protective cultures. There are also verified evidences about bioactive metabolites produced by these pro-functional microorganisms in food systems [1]. Although there are some promising strategies to manufacture PRO baked-goods such as microencapsulation, three dimensional printing, and direct utilization of thermophilic strains of PRO, while application of edible coatings as PRO carriers is a simple, cheap and efficient approach in bakery industries to manufacture functional products with potential health-promoting properties [20]. In general, survivability of PRO in complex food systems is associated with their potentials to tolerate the harsh conditions especially in terms of *aw*, pH and low nutrients. Moreover, presence of PRE components along with inherent resistance of PRO towards GIT environment affects viability of these beneficial microorganisms during their journey to the colon as the final destination [27]. It is also hypothesized that PRE activity of malt coating affects viability of PY in the produced cupcake. Furthermore, SD fermentation improved bioavailability of nutrients in produced SD added product due to the enzymatic activity of FBR to digest complex carbohydrates.

Textural characteristics

As shown in Table 1, crumb hardness significantly (P<0.05) increased by adding FBR and NFBR compared to the control in samples produced without malt coating. Moreover, control and NFRB added samples with malt coating had significantly higher crumb hardness compared to their corresponding samples without malt coating. Although FBR added samples showed higher porosity than those of the NFBR supplemented cupcakes, while their gumminess was significantly higher than the aforementioned samples. Furthermore, there was no significant difference among the produced cupcakes in terms of *aw*.

Table 1. Textural and quality features of the produced cupcakes supplemented with fermented black rice (FBR) and non-fermented black rice (NFBR) without/with malt coating (containing a potential probiotic yeast strain) compared to the control sample.

Cupcake samples	Crumb hardness (g)	Porosity (%)	Gumminess	*aw*
Control	1023.75 ± 39.34 b	61.45 ± 0.25 a*	746.17 ± 23.23 b	0.73 ± 0.01 a
FBR	1529.77 ± 34.54 a	49.80 ± 1.8 b*	962.14 ± 5.12 a*	0.68 ± 0.05 a
NFBR	686.50 ± 14.08 c	38.35 ± 0.35 c	466.02 ± 7.43 c	0.74 ± 0.01 a
Control + malt coating	1303.88 ± 83.43 B*	46.50 ± 0.9 A	822.45 ± 35.60 A*	0.70 ± 0.03 A
FBR + malt coating	1619.63 ± 19.64 A	41.95 ± 0.35 B	674.59 ± 4.68 B	0.70 ± 0.03 A
NFBR + malt coating	1235.73 ± 16.48 B*	36.75 ± 1.25 C	660.50 ± 57.48 B*	0.72 ± 0.02 A

Different uppercase and lowercase letters in each column indicate significant difference at P<0.05 among the produced samples with and without malt coating, respectively. Star symbol

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also shows significant difference between each produced sample coated with malt compared to the corresponding sample without malt coating, which was determined using *t*-test statistical analysis.

Application of chocolate coating reduced crumb hardness (2633.8 g) in the produced PRO cake in the study of Arslan-Tontul et al. (2019) compared to the control (2865.5 g) [21]. Meanwhile, application of 10% black rice instead of wheat flour resulted in increasing of crumb hardness compared to the control cake as reported by Mau et al. (2017), which was in line with our findings [28]. Although SD fermentation has undeniable effects on modification of textural features of the product, while mild acidic conditions obtained after SD acidification that is necessary for better enzymatic activity of SD microbial consortium and indigenous enzymes were not achieved in the present study. It is assumed that the high pH of yeasty fermentation compared to LAB acidification may be an important reason for this phenomenon. Moreover, textural features of the product are associated with physical destruction of the produced bubbles by bran particles of whole rice that resulted in reduction of specific volume in NFBR supplemented sample. Moreover, improper distribution of moisture and increasing of crumb hardness due to the presence of dietary fibers is another obstacle in the case of whole/high bran content flours. In addition, negative effect of gluten dilution on rheological behavior of the dough was also proposed as the key element for increasing of crumb hardness [4]. It is revealed that the higher gumminess in FBR added sample is also a key factor for its higher crumb hardness than those of the other produced cupcakes in the present study. Meanwhile, higher porosity of the SD added sample resulted in its acceptance from textural viewpoint.

Overall acceptability

As shown in Fig. 1, FBR and NFBR added samples showed significantly (P<0.05) lower OA than those of the control in both groups with or without malt coating. Meanwhile, there was no significant difference in terms of OA between FRB and NFBR supplemented cupcakes produced with or without malt coating. Moreover, comparison between each produced cupcake coated with malt and its corresponding sample without malt coating revealed significant difference between NFBR added samples.

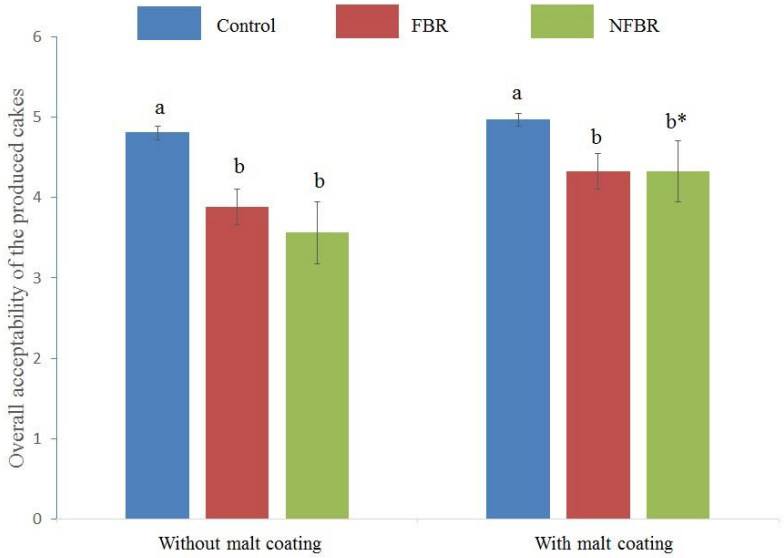

Fig. 1. Overall acceptability of the produced cupcakes supplemented with fermented black rice (FBR) and non-fermented black rice (NFBR) without/with malt coating (containing a potential

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probiotic yeast strain) compared to the control sample. Different letters in each group (with/without malt coating) indicate significant difference at $P<0.05$. Star symbol also shows significant difference between each produced sample coated with malt compared to the corresponding sample without malt coating, which was determined using *t*-test statistical analysis.

Among produced samples by Arslan-Tontul et al. (2019), chocolate coated PRO cake received higher OA score (4.2 out of 5-point hedonic scale) [21]. Zanjani et al. (2012) also reported that there was no significant difference between manufactured PRO cream-filled cake in comparison with the control sample in terms of OA [18]. Sensory attributes of the product may be affected by the SD microbial metabolites and/or bioconversion of the SD substrate into flavor active compounds. Direct effect of the substrate (black rice) on color, taste and chew-ability of the product is also another phenomenon affect OA. Moreover, high ash content of the whole black rice is responsible for hard crumb texture and subsequent improper mouth-feel of the product that are important parameters in OA and quality characteristics of the produced cupcake compared to the control sample. FoodOmics findings have also revealed the key role of microbial metabolites in sensory attributes of the SD supplemented products. There are several elements like dough yield (the ratio of dough to flour × 100), fermentation time and temperature, as well as back-slopping (addition a part of previously fermented SD to fresh dough-water mixture at specific time intervals) that affect microbial acidification, proteolysis and dynamic during SD fermentation. These factors are important in biodiversity of microbial consortia in this stressful fermented ecosystem, and they have crucial effects on type of produced metabolites and their complex interactions [4]. Overall, considering PRO potentials of predominant microorganisms isolated from stressful ecosystems, they can be used in production of functional PRO and/or synbiotic products with improved sensory attributes and textural properties [29-33].

4. Conclusions

Considering the importance of functional confectionary products in our daily diet, production of PRO baked goods has received considerable attention as an alternative for common snack foods. In the present study, FBR along with malt coating containing a potential PY were used in processing of a functional cupcake. Survivability of the studied PY after 6 days of storage was in an acceptable range. Although crumb hardness and gumminess of the FBR added sample was higher than those of the control cupcake, while its porosity and sensory attributes were acceptable. Accordingly, combined application of fermented pseudo cereals and adjunct PRO cultures is a promising way to manufacture a functional baked good.

5.Declaration of competing interest

This study was approved by the institutional review board of Gorgan University of Agricultural Sciences and Natural Resources. 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.

6.References

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چکیده

تولید فراورده‌های پروبیوتیک مبتنی بر غلات، یک راهکار مهم به منظور افزایش میزان مصرف این میکروارگانیسم‌های مفید در رژیم غذایی روزانه در سطح دنیاست. در مطالعه حاضر، ویژگی‌های بافتی، خصوصیات حسی و زنده‌مانی کشت همراه پروبیوتیک در کیک فنجانی حاوی برنج سیاه تخمیر شده با روکش خوراکی مالت مورد بررسی قرار گرفت. بر اساس نتایج، زنده‌مانی مخمر پروبیوتیک در روکش خوراکی مالت پس از ٦ روز نگهداری به ۱۰٦ پرگنه در هر گرم از محصول رسید. علاوه بر این، سفتی بافت (۱۵۲۹/۷۷ گرم) و صمغی شدن (۹٦۲/۱٤) در کیک حاوی برنج سیاه تخمیر شده به شکل معنی‌داری (P< ۰/۰۵) نسبت به نمونه شاهد بیشتر بود. با اینحال، تخلخل و پذیرش کلی مناسبی در نمونه مذکور مشاهده شد. بر این اساس، استفاده از مخمر پروبیوتیک در روکش خوراکی مالت به عنوان یک حامل مناسب، قادر به حفظ زنده‌مانی این کشت همراه در طی دوره ماندگاری کیک در محدوده پیشنهاد شده برای میکروارگانیسم‌های پروبیوتیک است. با توجه به قابلیت‌های سلامتی‌بخش پروبیوتیک‌ها و مقادیر مصرف کیک فنجانی در رژیم‌هایی غذایی امروزی، این محصول می‌تواند انتخاب مناسبی به منظور مصرف مقادیر بیشتر از میکروارگانیم‌های مفید باشد.

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