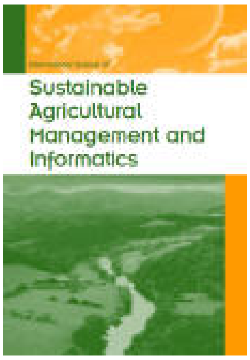

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Development of a novel digestive cookies' recipe valorising rice by-products serving circular bioeconomy

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Abstract: Rice production generates several by-product streams with limited valorisation in human consumption. Among them, rice bran is the most promising to serve circular bioeconomy. The current study presents the development of a novel digestive cookies (DC) recipe fortified with rice bran (RB), rice bran oil (RBO) and emmer flour. Fifteen formulations of DC were prepared and four of them were analysed for their antioxidant capacity and nutraceutical status. Results indicated that RB and RBO incorporation elevated total phenolics, ABTS and total flavonoids. Moreover, it decreased saturated and increased polyunsaturated, monosaturated fatty acids and dietary fibres, changes in energy profile. Furthermore, sensory evaluation showed a slight increased preference of the DC concerning aroma, texture, and crunchiness attributes concerns. Thus, a novel recipe of heathy functional RBDC can be produced. However, rice industry should consider investing in the production of stabilised RB following the regulations for a product designated for human consumption.

Keywords: by-products; rice; bran; oil; emmer; phenolics; flavonoids; ABTS; sensory panel.

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Michalis Ioannou is the owner of the confectionery which was established in 1952. He leads the R&D of the company creating and developing all the new recipes and products and improving the old traditional ones. The factory provides a variety of products for the Greek and the international markets such as USA.

Paschalis Lithoxopoulos is an agronomist specialised in food science of sweets, pastries and ice creams employed at Ioannou confectionery. For many years, he was managing the production and supply line of the factory and in parallel he was working on the R&D department for the development of new products of the factory.

Dimitris Katsantonis is the Director of Research Hellenic Agricultural Organization-DEMETER, working on rice department since 1999. His research interests focus on technology of agro-nutrition and especially functional foods based on rice and its by-products, digital agriculture, remote sensing, agronomy, and plant pathology. He has an h-index 16 in WoS and 18 in Scopus.

1 Introduction

Brown rice belongs to the most nutritious cereals and is considered a rich matrix of numerous bioactive compounds. Rice bran (RB) represents approximately more than 10% of the whole rice grain (Wang et al., 1999; Akter et al., 2020), mainly composed of the embryo, pericarp, and 20% of rice bran oil (RBO) (Mohamed et al., 2016).

RB contains mostly protein (11%–17%), complex carbohydrates and dietary fibres (DF) (6%–14%), fat (14%–15%), essential minerals along with antioxidants, vitamins, phytosterols and oryzanol (Yadav et al., 2011; Gul et al., 2015; Irakli et al., 2018; Akter et al., 2020; Bultum et al., 2020). RB remains mainly unavailable for humans due to the rapid rancidity of free fatty acids (Mohamed et al., 2016; Irakli et al., 2018). However, RB can be utilised in the food industry due to its bioactive compounds that play a protective role against certain diseases and types of cancer (Bultum et al., 2020). Nowadays, RB is mainly used as animal feed which may result in a 40% nutrient loss from human consumption (Nugrahani et al., 2019; Bultum et al., 2020). It can be used as an ingredient in fertilisers (Kadoglidou et al., 2019) and in biofuel formulations (Akter et al., 2020; Bultum et al., 2020). Recent studies indicate that RB stabilising methods can be adopted by industry to improve the perspective of its use as a supplement in food and in the pharmaceutical industry (Sharif et al., 2014; Irakli et al., 2018; Bultum et al., 2020). Thus, it could be used in novel functional food products suitable for daily consumption, such as bread (Irakli et al., 2019), sponge cakes, biscuits, and cookies (Bultum et al., 2020).

RBO has a nutritional profile rich in bioactive compounds and antioxidants, with content of approximately 90% triglycerides, as well as unsaturated fatty acids (UFA) and saturated fatty acids (SFA), including oleic and linoleic acid as the most dominant ones (Mohamed et al., 2016; Nugrahani et al., 2019). RBO can act as a protection factor against chronic diseases such as heart diseases, hyperlipidemia, platelet aggregation and certains types of cancer (Xu et al., 2001; Cicero and Gaddi, 2001; Hudson et al., 2000; Mohamed et al., 2016; Eleftheriadis et al., 2019).

Emmer flour (EF) derives from Triticum turgidum subsp. dicoccum (Schrank ex Schübl.) Thell. belongs among the ancient wheat genotypes (Kissing Kucek et al., 2017; Khmeleva et al., 2021). However, due to low yields it was gradually abandoned and replaced by the modern wheat varieties. EF is known for its high protein content (PC) (12%–25%), vitamins, minerals, DF and polyunsaturated fatty acids (PUFAs) (Longin et al., 2016; Khmeleva et al., 2021). Nowadays, there is a perceptible trend for consumers to turn to the consumption of old wheat genotypes with better sensory and nutritional properties. Besides, there is a socioeconomic trend, where farmers prefer to cultivate emmer in regions of low production intensity, supporting the orientation of artisan bakeries, which attempt to compete with big bakery product industries (Longin et al., 2016). Kissing Kucek et al. (2017) analysed the consumers demand for food products provided by local farmers, of less additives and biological and organoleptically acceptable. For these reasons consumers present a tendency to purchase flour from small scale local mills, resulting in EF elevated demands. In the past decades, the consumption of products enriched with cereals' DFs and bioactive compounds has been promoted. These trends can lead to potential health benefits for various chronic diseases like metabolic syndrome, hypertension, heart diseases, diabetes mellitus, chronic gastrointestinal disorders (irritable bowel syndrome), and specific type of cancers (Longin et al., 2016; Martinez-Saez et al., 2017; Liu et al., 2019; Akter et al., 2020). Therefore, the adoption of a low chain carbohydrates diet is the best way to improve their wellbeing and the control of the symptoms (Gibson and Shepherd, 2010). From the bakery products available in the market, cookies and biscuits are most widely consumed as quick, palatable, and ready-to-eat snacks (Jia et al., 2020). Several important factors affect the general quality of the cookies, such as the quality of raw materials, flour, sugar, fat, and rice milk (RM), gathering the main interest, hence kneading, baking, and cooling conditions of the formulation procedures (Tavares et al., 2016; Jia et al., 2020). However, organoleptic characteristics such as taste, odour and flavours, mouthfeel, aftertaste, total acceptance, and the presence of a commercially significant extended shelf life ready-to-eat product, are strong assets in evaluating the preference for general nutritional needs of consumers (Tavares et al., 2016; Hoang at al., 2022; Ribeiro et al., 2023).

The aim of the current study was to develop a new digestive cookie (DC) recipe, competent to combine the important properties of the EF with rice by-product such as RB, RBO and RM. Furthermore, to determine the nutritional value, the antioxidant capacity, and the sensory factors, which can characterise the fortified RB DCs as novel functional products.

2 Materials and methods

2.1 Procurement of raw materials

The commercial EF was supplied from the local milling industry. The salt, the granulated sugar, the baking powder, the butter, and the pasteurised RM were purchased from the local market. RB was obtained from paddy rice grown in the rice fields located at Kalochori Experimental Station of the Institute of Plant Breeding and Genetic Resources (IPG&RB) (Greece). The grains were dehulled using the rice dehulling mill (TakaYama MTH-35A, Taiwan) and polished using a whitening mill (Satake, Testing Mill TM05C, Japan) to collect fresh RB. Then the obtained RB was heat stabilised according to the method described by Irakli et al. (2018), packed, vacuum sealed in aluminium bags and kept in the freezer at –20ºC. The RBO manufactured by the supercritical carbon dioxide extraction method, was obtained from a local Greek retailer.

2.2 Chemical Reagents

Folin-Ciocalteu, Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), gallic acid (GA), Rutin (RE) used for the study were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.3 Cookie formulations

DC formulations were developed depending on three factors: RB, RBO and the particle size (PS) of RB: 0.5 mm (PS05) and 1.0 mm (PS10). All formulations are presented in Table 1.

2.4 Cookies preparation

The main dough formulation on 100 g flour basis consisted of EF (48.0 g), sugar (19.2 g), baking powder (0.4 g), salt (0.4 g), butter (19.2 g) and RM (12.8 g). The doughs were mixed in a three-step procedure with a kitchen-aid mixer (Kenwood Major Titanium, KMM023, 1,500-Watt) for 3 minutes. The dry ingredients (flour, salt, sugar, baking powder) were initially homogenised and then the shortenings were added. The formulation was creamed with the mixer for 1 min at low speed. The mixing continued after adding the RM for 2 min (1 min at low speed and 1 min at high speed). The dough was then gently scraped from the mixing bowl, hand-kneaded into rounded balls and placed into the fridge for 10 min to be firm. The dough was sheeted to a thickness of 0.5 cm and cut into round shapes using a six cm diameter dough cutter. Then it was transferred onto aluminium trays and baked for 16 min at 180ºC. After baking, the cookies were cooled down at room temperature (20º–21ºC) and stored for further analysis.

Table 1 Dough formulations of DC (on a 100 g flour basis)

Formulations	*RB particle size (mm)*	*Ingredients (g)*									
		EF	*RB*	*EM/RB substitution (%)*	*Butter*	*RBO*	*Butter/RBO substitution (%)*	*Sugar*	*Baking powder*	*Salt*	*Rice milk*
T01 (control)		48	0.0	0	19.20	0.0	0	19.2	0.4	0.4	12.8
T02			0.0	0	18.24	0.96	5				
T03			0.0	0	17.28	1.92	10				
T04	0.5	40.8	7.2	15	19.20	0.0	0				
T05			7.2	15	18.24	0.96	5				
T06			7.2	15	17.28	1.92	10				
T07		33.6	14.4	30	19.20	0.0	0				
T08			14.4	30	18.24	0.96	5				
T09		30.6	17.4	36	16.90	2.3	12				
T10	1.0	40.8	7.2	15	19.20	0.0	0				
T11			7.2	15	18.24	0.96	5				
T12			7.2	15	17.28	1.92	10				
T13		33.6	14.4	30	19.20	0.0	0				
T14			14.4	30	18.24	0.96	5				
T15		30.6	17.4	36	16.90	2.3	12				

Notes: RB = Rice bran, EF = emmer flour, RBO = Rice bran oil

2.5 Sensory evaluation

Sensory analysis of DCs was performed by a group of 13 semi-trained panellists (3 males and 10 females, aged 35 to 55), belonging to the staff of the Institute of Plant Breeding and Genetic Resources (IPB&GR), the Hellenic Agricultural Organization – ‘DEMETER’. The panellists did not have any health problems or allergies that could compromise the evaluation process. Water was provided to rinse the mouth between evaluations. The sensory attributes were: general appearance (colour uniformity/density /overall acceptance), texture (oiliness, graininess, crunchiness and mouthfeel), DC character (flavour/odour, taste, aftertaste) and overall acceptance using the 9-point hedonic scale (Wichchukit and O’Mahony, 2015).

2.6 Colorimetry evaluation

The colour of the cookies was determined using a HunterLab chromameter (MiniScan XE Plus, Reston, USA). The colour model was the CIEL*a*b* with the values of $(0 < L^* < 100)$, $(128 < a^* < 127)$ and $(128 < b^* < 127)$ representing lightness (L^*), redness (a^*), and yellowness (b^*), respectively.

2.7 Chemical composition

The content of moisture and ash was determined according to AACC method 44-15A (AACC, 2000e) and 08-01, (AACC, 2000a). PC in RB and EF was determined according to the AOAC method 979.09 (AOAC, 1990) and in cookies according to the AOAC method 950.36 (AOAC, 1990). Fat content was determined according to AACC method 30-10 (AACC, 2000c), while the profile of fatty acids (FA) was analysed using a gas chromatography-flame ionisation detector (GC-FID) (Omar and Salimon, 2003). DF were determined according to AACC method 32-05 (AACC, 2000d). Carbohydrate content was estimated by subtracting the sum of the remaining nutrients [Carbohydrate (%) = 100% − % (Moisture + Ash + Protein + Fat + FD)]. Samples were defatted (Wang et al., 1999) prior to determine the total phenolic content (TPC) according to Irakli et al. (2019). The total flavonoids (TF) content was determined following the method described by Bao et al. (2005). ABTS assay (Irakli et al., 2019) was used to determine ABTS radical scavenging activity. The energy value was calculated with total carbohydrates (including fibres) and expressed as kcal/100 g (Jiang et al., 2014).

2.8 Physical characteristics

The dimensional analysis, width, and thickness were carried out according to the official method as described by AACC method 10.50.05 (AACC, 2010) and repeated four times for each measurement. The spread ratio was evaluated using the formula 10*Width/Thickness measuring six cookies according to AACC Method 10-50D (AACC, 2000b).

2.9 Statistical analysis

Data were subjected to ANOVA and means were compared with the least significant difference test (LSD) at a 5% level of probability was calculated to carry out the mean comparisons. Difference among means was compared using Duncan's multiple range test at significant level 95% ($p \leq 0.05$). All statistical analysis was performed using IBM SPPS Statistics v23 software package.

3 Results and discussion

3.1 Selection of formulations

A primary sensory evaluation was performed to select the most preferable formulations (Table 2). Evaluation of colour ranged from 6.42 (T9) to 7.75 (T11), for Mouthfeel from 3.67 (T7) to 7.08 (T12), odour/flavour 4.17 (T15) and Taste from 3.08 (T15) to 7.00 (T3, T10). More importantly, Overall Acceptance ranged from 2.5 (T15) to 7.17 (T10). Results showed that RB incorporation should not exceed the 15% substitution of EF. In general, the addition of higher RB concentration had a negative impact on sensory parameters. Furthermore, PS05 revealed less preference compared to PS10, since average Overall Acceptance was for PS05 4.5 and for PS10 5.2. Furthermore, after thorough study of the evaluation results four formulations belonging to PS10 group were appeared as the most preferable and promising ones for further analysis: T01 (control), T03 (0% RB with 12 % RBO) T10, T11, T12 (15% RB with PS10).

Table 2 Sensory evaluation analysis of colour, mouthfeel, door/flavour, taste and overall acceptance attributes for optimum formulations of DCs

Formulations	*Colour*	*Mouthfeel*	*Odour/flavour*	*Taste*	*Overall acceptance*
T01 (control)	7.42 ± 0.42*	5.92 ± 1.68	5.75 ± 0.54	6.08 ± 0.48	5.83 ± 2.04
T02	7.17 ± 0.61	5.83 ± 1.40	6.17 ± 0.34	6.50 ± 0.26	5.58 ± 1.98
T03	7.25 ± 0.37	6.83 ± 1.40	6.50 ± 0.54	7.00 ± 0.51	6.83 ± 1.59
$T04_{PS05}$	7.42 ± 0.34	4.67 ± 1.83	5.75 ± 0.57	5.58 ± 0.51	4.92 ± 1.98
$T05_{PS05}$	6.67 ± 0.28	6.08 ± 1.51	5.42 ± 0.34	5.92 ± 0.52	6.08 ± 1.31
$T06_{PS05}$	6.67 ± 0.56	6.67 ± 1.56	6.50 ± 0.36	6.50 ± 0.26	6.17 ± 0.94
$T07_{PS05}$	6.83 ± 0.52	3.67 ± 1.78	4.75 ± 0.46	3.92 ± 0.47	3.58 ± 1.51
$T08_{PS05}$	7.58 ± 0.45	4.50 ± 1.68	5.17 ± 0.60	5.25 ± 0.54	3.83 ± 1.59
$T09_{PS05}$	6.42 ± 0.56	3.83 ± 1.99	3.58 ± 0.65	3.42 ± 0.47	2.58 ± 1.73
$T10_{PS10}$	7.08 ± 0.45	7.08 ± 1.62	6.67 ± 0.43	7.00 ± 0.54	7.17 ± 0.94
$T11_{PS10}$	7.75 ± 0.25	6.17 ± 1.47	5.33 ± 0.57	6.00 ± 0.44	6.33 ± 1.61
$T12_{PS10}$	7.08 ± 0.45	7.08 ± 1.38	6.67 ± 0.45	6.92 ± 0.43	6.42 ± 1.38
$T13_{PS10}$	6.67 ± 0.56	5.50 ± 1.98	6.25 ± 0.48	5.92 ± 0.43	5.17 ± 1.90
$T14_{PS10}$	7.75 ± 0.33	5.17 ± 0.59	5.83 ± 0.52	4.92 ± 0.40	4.00 ± 0.52
$T15_{PS10}$	6.75 ± 0.49	3.75 ± 2.09	4.17 ± 0.64	3.08 ± 0.60	2.50 ± 1.45

Notes: PS05 (particle size 0.5mm) and PS10 (particle size 1.0mm)
*Values are expressed as mean ± standard deviation.

3.2 Chemical characteristics

The physicochemical characteristics of RB, EF and DC are presented in Table 3. All samples showed moisture content lower than the allowed maximum limit (4.5 g/100 g) established by the WHO and the FAO (FAO/WHO, 2001). Similarly, Bultum et al. (2020) reported that moisture content decreases as a result to DF elevation in the dough mixture of the biscuits due to substitution of the gluten content with RB. Akter et al. (2020) concluded that water absorption ability was the factor of increased moisture after 5% RB protein was added in the biscuit mixture.

Ash content was significantly higher in T12, T11 and T10, compared to the control, showing the impact of RB incorporation in the DCs. Moreover, fat content was slightly increased in T12, which incorporated the highest ratios of RB and RBO, while the rest did not different significantly. Similarly, to our results, several authors reported ash content elevation due to DF increases after RB addition (Bultum et al., 2020; Akter et al., 2020; Jiamjariyatam, 2022).

SFA were lower in T03 and T12, the formulations with the higher substitution of butter with RBO (10%), showing a positive impact. Moreover, monounsaturated (MUFA) and PUFAs appeared to be higher in T12, 7.7% and 5.7%, respectively, the formulation with the highest RB incorporation and the highest butter substitution from RBO, while control appeared with the lowest values (5.9% and 4.1%), the treatment with the highest amount of butter.

Furthermore, PC did not appear to be influenced by the RB and RBO incorporation, since the mean values were close to the overall mean (9.1%). Results of elevated PC in biscuits prepared with 5% RB, 14% GBRF, 20% RB and 50% black rice flour (BRF), were also reported by Akter et al. (2020), On-Nom et al. (2016), Bultum et al. (2020) and Jukić et al. (2022).

Additionally, the presence of RB and RBO in the highest substitution of EF and butter in T12, appeared to significantly reduce the carbohydrates content 3.8% compared to the control. Similar results reported by Bultum et al. (2020) in biscuits incorporated with up to 30% RB.

Also, DF appeared to be significantly increased (1.2%) by RB incorporation compared to the control. Yadav et al. (2010) presented 0.3% DF increases in biscuits after 15% defatted rice bran (DRB) incorporation. Similarly, DF elevations were reported after a significant increase of RB (10% and 20%), in RB fortified biscuits, bread and RB wax biscuit sticks (Bultum et al., 2020; Irakli et al., 2018; Jiamjariyatam, 2022). Moreover, On-Nom et al. (2016) reported 3.68% DF increase in cheese shake biscuits due to the brown rice germination.

Finally, energy profiles were not affected by RB and RBO, since all means were very close to the overall mean of 456.2 kcal/100g.

3.3 Total phenolic, flavonoids and ABTS

TPC was significantly increased after RB and RBO addition (Table 4). From 46.6 mg GA/100g in the control elevated to 117.8 GA/100g in T12, an almost 2.5-fold increase. Moreover, significant TPC elevation appeared in T11 (101.0 GA/100g) and in T10 (95.2 mg GA/100). However, when only RBO was incorporated, TPC reached the 52.9 mg GA/100 in T03. Irakli et al. (2018) reported that the TPC was increased due to the high amounts of phenolic compounds present in the raw material in bread fortified with 30% of RB. Croitoru et al. (2018) after analysing TPC in muffins formulated with BRF, reported a reduction of the total antioxidant capacity after baking due to the heat sensitivity of the TPC. Moreover, BRF incorporation increased TPC, starting from 0% BRF 64.4 mg to 170.4 mg (50% RBF) to 226.5 mg (100% RBF).

Furthermore, control samples had ABTS value of 92.5 mM TE/100g. RB and RBO addition increased ABTS more than 2.5-fold in T12 (257.3 mM TE/100g), 229.0 mM TE/100g in T11 and 212.5 TE/100g in T10. However, when only RBO was incorporated in T03 the increase was significantly smaller (113.8 mM TE/100g) compared to the other ones. Similarly, Irakli et al. (2018) reported ABTS elevations in bread fortified with RB. Croitoru et al. (2018) presented elevated antioxidant activity, when the basic formulation was fortified with at least 50% of BRF (445.89 mM TE/100 g).

Furthermore, FL analysis determined high elevations in the DCs with RB and RBO (Table 4). The FL value of the control increased from 4.3 mg RE/100g to 44.0 mg RE/100g in T12 and 40.6 mg RE/100g in T10. T03 followed the same trend as the rest chemical traits since it RBO presence appeared not to influence FA in comparison to the control.

Table 3 Chemical characteristics of the DC's formulations and raw materials

Formulations	*Moisture (%)*	*Ash (%)*	*Fat (%)*	*Saturated fatty acids (%)*	*Monounsaturated fatty acids (%)*
T1 (control)	4.2	1.9	18.1	8.2	5.9
T03	4.3	1.8	18.1	6.7	6.8
T10	4.1	2.3	19.0	8.1	6.3
T11	4.0	2.3	18.7	7.3	6.6
T12	4.3	2.4	20.3	6.9	7.7
Mean	4.2	2.1	18.8	7.4	6.7
σ	*0.1*	*0.2*	*0.8*	*0.6*	*0.6*
Raw materials					
Emmer flour	*5.5*	*1.8*	*2.4*	*0.3*	-
Rice bran	*6.2*	*8.3*	*20.4*	*2.8*	*6.7*
Rice bran oil	-	-	*98.5*	*22.2*	*41.8*

Formulations	*Polyunsaturated fatty acids (%)*	*Proteins (%)*	*Carbohydrates (%)*	*Dietary fibres (%)*	*Energy (kcal/100 g)*
T1 (control)	4.1	9.1	62.7	3.0	455.9
T03	4.6	8.9	61.5	3.0	450.2
T10	4.7	9.4	60.9	4.2	461.0
T11	4.8	8.9	59.9	4.2	451.3
T12	5.7	9.0	58.9	4.2	462.7
Mean	4.8	9.1	60.8	3.7	456.2
σ	*0.5*	*0.2*	*1.3*	*0.6*	*5.0*
Raw materials					
Emmer flour	-	*12.5*	*70.3*	*2.7*	*344.1*
Rice bran	*6.6*	*17.1*	*16.7*	*20.1*	*384.9*
Rice bran oil	*38.1*	-	-	-	*1223.0*

Note: σ = Standard deviation of the means

Overall, there is a clear trend when both RB and RBO were incorporated together in the DC formulations, which significantly elevated the antioxidant capacity, thus, producing healthier functional products. Although only RBO incorporation was not enough to elevate antioxidant profile. Jukić et al. (2022) reported a positive effect of DF to TPC, when Hulless Barley Flour was incorporated in biscuits. They concluded that the antioxidant capacity is elevated through the TPC increase and baking manipulations leading to the release of bound phenolic compounds through caramelisation and Maillard reactions. Similarly, Pinto et al. (2023) concluded that phenolic content released carbohydrate and amino acid reactions in high temperature baking conditions of functional cookies enriched with chestnut shells.

Table 4 Results of TPC, ABTS and FLs analysis of DC

Formulations	*TPC (mg GA/100g)*	*ABTS (mM TE/100g)*	*FLs (mg RE/100g)*
T1 (control)	46.6[e]	92.5[e]	4.3[b]
T03	52.9[d]	113.8[d]	5.6 [b]
T10	95.2[c]	212.5[c]	44.0[b]
T11	101.0[b]	229.0[b]	40.6[b]
T12	117.8[a]	257.3[a]	44.0[a]
LSD	3.59	1.02	4.08
CV%	3.21	0.41	9.05

Notes: TPC = total phenolic content, ABTS = 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate), FL= flavonoids, LSD = least significant difference, CV% = coefficient of variation, means followed by different letters in the same column are significantly different ($p \leq 0.05$), CV = coefficiency of variance

3.4 *Physical characteristics of the DC*

The physical characteristics of the DC are presented in Table 5. Generally, the mean value of the DC Weight decreased with the increase of RB incorporation but with insufficient results. Yadav et al. (2010) presented cookie weight increases up to 14.5% after adding RB. They concluded that this increase was result due to the ability of the DF to absorb water during kneading and to retain the oil portion during the baking process, which could also lead to excessive moisture loss. Similar results were also presented by Irakli et al. (2018), Jia et al. (2020) and Jukić et al., (2022).

DC Width measurements were identical in all formulations (6.0 cm) (Table 5). However, width decreases, and firmer products were reported by Yadav et al. (2010) and Mohamed et al. (2016) following an addition of 15% RB in biscuits. Akter et al. (2020) and Jiamjariyatam (2022) reported increase of the biscuit width when RB was added. They concluded that these increases were due to oil retention during baking that negatively influenced the gluten network and led to a less firm biscuit.

Furthermore, %RB and %RBO did not affect the DC Thickness in all formulations (Table 5) since the cookies appeared with uniform rising with 0.8 cm height. Similarly, after RB incorporation marginal decreases were reported by Yadav et al. (2010) and Akter et al. (2020), while higher decreases were reported by Mohamed et al. (2016).

High or a low spread ratio affects the softness or hardness of the cookies and higher spread ratio elevates the desirability of the biscuit (Bose and Shams-ud-Din, 2010; Handa et al., 2012; Raymundo et al., 2014; Drakos et al., 2019). According to Table 5, RBO incorporation without RB, elevated the spread ration in T03 (80.5), an increase which is significantly higher than the control, baked with no butter (76.2). Furthermore, T12 and T10 appeared with significant lower spread ratio and T11 with not significant differences in comparison to the control. Although, RB addition significantly decreased spread ratio. The spread factor of cookies is affected by the competition of ingredients for the available water; flour or any other ingredient that absorbs water during dough mixing will decrease it (Yamsaengsung et al., 2012). Bose and Shams-ud-Din (2010) and Yadav et al. (2010) concluded that the finer milled flours used resulted to a higher spread ratio. According to Prasanth Kumar et al. (2014), an up to 20% of RBO addition can increase of the cookies' spread ratio. Jia et al. (2020) reported that the increase of the DF leads to an elevated water holding capacity that helps the dough to hold more water and display better gelatinisation ending to softer biscuits. Jukić et al. (2022) reported that the increase of hulled barley flour in the short-dough cookies mixture negatively influences both width and spread ratio due to dough viscosity elevation endorsed by the more water holding capacity, which doesn't allow the gelatinisation process to fully develop concluding to a firmer, crispier, and hard to consume biscuit.

Table 5 Effect of composition on the physical characteristics of the DC' formulations

Formulations	*Weight (gr)*	*Width (cm)*	*Thickness (cm)*	*Spread ratio (W/T)*
T01 (control)	14.67^{a}	6.0	0.8	76.2^{b}
T03	14.10^{bc}	6.0	0.8	80.5^{a}
T10	13.80^{c}	6.0	0.8	73.5^{c}
T11	14.27^{b}	6.0	0.8	75.0^{bc}
T12	14.23^{b}	6.0	0.8	73.0^{c}
LSD	0.36	-	-	2.1
CV%	1.89	-	-	2.05

Notes: Means followed by different letters in the same column are significantly different ($p \leq 0.05$), CV = coefficiency of variance

3.5 Colour characteristics

Colour characteristics of the selected formulations are presented in (Table 6). The highest L* value appeared in the control, while there is a clear pattern that RB incorporation decreased L* in all formulations, with highest value in T12, (darker cookies). Bultum et al. (2020) reported that in full fat 15% RB addition in biscuits the L* values declined from 65.9 to 43.95. Generally, the addition of DF is the main reason of a baked product with darker surface, and it is due to caramelisation and Maillard reaction between RB, protein, and carbohydrates of the dough formulation (Jia et al., 2020; Jiamjariyatam, 2022; Jukić et al., 2022). On the contrary, red colour (a*) was elevated 2% approximately by the presence of RB in T12, T11 and T10, while RBO addition shows a clear pattern that did not influence redness, since there are no significant differences between T03 (no RBO) and the control. Jia et al. (2020) reported an increase in a* following a RB addition from 0% (5.77) to 15%RB (13.65) and 25%RB (18.1). In contrast, On-Nom et al. (2016)

reported a* decrease following a 14% GBRF incorporation in cheese shake cookies (a* = 11.76). Similarly, yellowness was elevated by the presence of RB, with no significant differences between T12, T11 and T10, while control formulation cookies had slightly less b* value (1.5%) than T03 (no RB). However, Bultum et al. (2020) and On-Nom et al. (2016) reported lower b* values in GBRF biscuits. Our results partially agree with Mohamed et al. (2016), who reported RB biscuits with decreased L* and increased a* and b* values. The same authors concluded that the darker colour was also influenced by the addition of other ingredients that may interact with the RB during baking, elevating Maillard reactions resulting to darker baked products (Nanditha and Prabhasankar, 2009; Jukić et al., 2022), but also due to the phenolic compounds oxidation while kneading (Pasqualone et al., 2017; Irakli et al., 2018).

Table 6 Effect of composition on the colour characteristics of DC

Formulations	*L**	*a**	*b**
T01 (control)	70.8^{a}	10.5^{b}	27.1^{c}
T03	70.6^{a}	10.7^{b}	28.6^{b}
T10	69.5^{b}	12.1^{a}	32.0^{a}
T11	68.3^{c}	12.2^{a}	32.0^{a}
T12	67.1^{b}	12.5^{a}	32.2^{a}
LSD	1.05	0.45	0.64
CV%	1.12	2.89	1.56

Notes: Means followed by different letters in the same column are significantly different ($p \leq 0.05$), CV = Coefficiency of variance

3.6 *Sensory evaluation*

The results of the organoleptic evaluation of the DC appearance are presented in Table 7. The three parameters are mainly used together as indicators of the panel preference tendency, while individually cannot define consumer preference and desire. The colour density attribute evaluates whether the biscuit dough prepared has been properly knead and fermented to develop a colour consumer acceptable.

Table 7 Sensory evaluation analysis of Colour attributes of DC

Formulations	*Density*	*Uniformity*	*Overall appearance*
T01 (control)	4.50 ± 0.58	8.50 ± 0.26	7.42 ± 0.42
T03	6.58 ± 0.50	7.42 ± 0.36	7.25 ± 0.37
T10	7.42 ± 0.38	6.50 ± 0.54	7.08 ± 0.45
T11	6.50 ± 0.36	7.75 ± 0.35	7.75 ± 0.25
T12	7.33 ± 0.36	6.67 ± 0.47	7.08 ± 0.45

Notes: *Means were calculated as average of 5 cookies ± Standard deviation

Results in Table 7 show that according to the density evaluation, all formulations were more preferable than the control, while uniformity showed almost reverse results. However, the overall appearance evaluation showed a small no significant preference in T11, while in general the panel showed the similar preference to all biscuits compared to the control. On-Nom et al. (2016) reported that 14% GBRF addition in cheese shake biscuits gave higher panel preference. In other studies, the colour and aroma preference in RB DC were slightly decreased when %RB was increased from 8.34 (0% RB) to 8.01 (25% RB) and from 8.31 (0% RB) to 8.02 (25% RB) (Bultum et al., 2020). Similarly, RB incorporation in DRB biscuits resulted to a colour preference decrease from 8.7 in 0%RB to 7.0 in 15% RB (Yadav et al., 2010). However, when low levels (up to 5%) of RB were added into biscuits no differences in panel preferences were reported by Akter et al. (2020).

Table 8 Sensory evaluation analysis of texture and aromatic attributes of DC

Formulations	*Texture*		
	Oiliness	*Graininess*	*Crunchiness*
T01 (control)	5.25 ± 0.58*	5.50 ± 0.50	5.83 ± 0.55
T03	5.83 ± 0.67	6.42 ± 0.72	7.75 ± 0.28
T10	5.58 ± 0.57	6.33 ± 0.78	7.75 ± 0.48
T11	6.08 ± 0.34	6.17 ± 0.41	7.17 ± 0.32
T12	5.83 ± 0.56	6.33 ± 0.72	8.17 ± 0.30

Note: *Means were calculated as average of 5 cookies ± Standard deviation

The texture of the cookies and bakery products is a major component in their evaluation as snacks. The oiliness, graininess and crunchiness indicate the sense of oil during rating, the sense of flour excess, or the sense in the palate while chewing and swallowing the crumbs (Table 8). Oiliness was slightly affected by the incorporation of RB and RBO compared to the control cookies, which appeared with the lower evaluation (5.25). However, the Graininess evaluation appeared with a clear trend, since all formulations were very close among them, with highest score in T03 (6.42), while scores were greater than the control. Similar trend appeared in the crunchiness, where every formulation gave significantly higher evaluation than the control, while the highest score appeared in T12 (8.17).

4 Conclusions

The DC fortified with RB and RBO were acceptable and more nutritious than the cookies produced with only EF. Substitutions of EF with RB up to 15% levels significantly increased the antioxidant activity and nutraceutical status of the DC without any negative effect on the sensory attributes. TPCs, ABTS and particularly total flavonoids were greatly elevated. RB cookies were determined by greater total dietary fibres, while the energy profile remained unchanged. The butter substitution with RBO up to a level of 10% induced the positive effect of reduction of SFA and a simultaneous increase of polyunsaturated and monounsaturated fatty acids, leading to the production of healthier cookies. Colour attributes were affected by darkening of the RB fortified cookies, while in the case of density, uniformity and overall appearance were negatively affected by RB and RBO incorporation. Finally, sensory analysis of the Taste, Odour/flavour, Overall Appearance, and the texture attributes showed slight preferences in the higher RB and RBO incorporations, especially in the case of crunchiness. The current study reveals that rice by-products, can be used in the baking industry of healthier and functional snacks as nutritional improving factors. However, rice milling industry needs investments to improve the conditions of the production of certified and stabilised RB designated for human consumption. This is the important pathway to increase the valorisation of a product that until nowadays it comprises mainly in the animal feed process.

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