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Functional Properties and Nutritional Quality of Processed Cheese Spreads Enriched with Black Rice Powder

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BLACK rice has been reported to contain many bioactive compounds such as protein, crude fiber, total carbohydrates and minerals with attractive purple color making it a valuable component in dairy industries. Partial replacement of dairy ingredients by 2-6% (2.06, 4.12, and 6.18 kg100/kg) black rice powder (BRP) to make functional processed cheese spreads (PCS) was investigated. Chemical composition, microbiological, rheological characteristics, total phenolic compounds (TPC), antioxidant activity (AA) and sensory evaluation of PCS were determined. Results showed that no significant difference was observed in dry matter and fat content between PCS and control sample (p>0.01), however, the protein content was significantly decreased by the addition of BRP (p<0.01). Adding different ratios of BRP in PCS increased the TPC and AA than the control sample as fresh and throughout the cold storage period. Furthermore, fortification with BRP significantly affected the rheological characteristics of PCS. The inclusion of black rice powder at different concentrations in the PCSs mix had no noticeable effect on the microbiological quality (p>0.01). Sensory evaluation results revealed that using 2% BRP in PCS processing had higher acceptability compared to control sample. Thus, BRP could be incorporated in PCS to develop a product with acceptable functional, nutritional and sensory properties.

Keywords: Black rice, Chemical composition, Processed cheese spreads, Antioxidant activity, Rheological characteristics, Sensory properties.

Introduction

Functional foods are defined as foods that promote health by reducing the risk of diseases or by enhancing the immune response. Functional foods should be consumed as part of a "normal' diet without consuming unusual quantities of a given food (Pratiwi and Purwestri, 2017). Traditional processed cheese is obtained by mixing natural cheeses of different ages and degrees of maturity in the presence of emulsifying salts and other dairy and nondairy ingredients under the influence of heat and agitation to form homogenous product with extended shelf life (Hladká et al., 2014). The utilization of other ingredients in processed cheese production has opened up a wide range of food products with enhanced functional properties. All these substitutes including protein-based, fat-based and carbohydrates-based will lead to decrease production cost, provide flavour or texture, or improve the shelf life (Kapoor and Metzger, 2008). According to the updated version of Egyptian Organization for standard, the dry matter should not less than 36% and the fat/ dry matter not less than 35% and not more than 65% for full fat processed cheese spreads. In the updated version of Egyptian Organization for standard and quality of processed cheese (ES:999/2013) (Egyptian Organization for Standards and Quality, 2013), the producer of processed cheese has a choice to use any food stuffs up to 15% to raise the total solids. In addition, there was no limit for lactose and no minimum percentage of protein in cheese.

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In order to obtain a suitable balance between flavour and texture in processed cheeses, cheeses are selected for use depending on their type, flavour, composition (moisture content, fat, protein, and calcium), ripening (degree of proteolysis), consistency, and its pH value. A good processed cheese must be smooth, homogenous, uniform in color and free of holes formed by fermentation. Furthermore, the quantity of emulsifying salt used also plays an important role in the functionality and aspects of the final product and can compose up to 5% of the total mixture (Tamime, 2011).

Black rice (BR) is a type of rice species (*Oryza sativa* L.) mainly cultivated in Asia, which is gluten free, with high nutritive value (Chung et al., 2014). It is one of the most potential plant sources of dark purple color of anthocyanin pigments. Its kernel contains high levels of bioactive compounds such as γ-oryzanol and phenolic compounds including anthocyanin. These compounds had been found to promote human health such as reducing the low-density lipoprotein cholesterol (LDL), improving lipid profile, anti-inflammatory, antioxidant activity (Guo et al., 2007 and Pratiwi and Purwestri, 2017). Among these compounds, dark purple anthocyanin pigments contribute both attractive color and strong antioxidant activity (Loypimai and Moongngarm, 2019).

BR is a whole grain with highly nutrients contents among rice types, higher fiber content, anthocyanins, vitamins niacin, thiamine, B and E, iron, magnesium and phosphorous. It contains more protein, minerals and dietary fibers than brown and white rice (Ichikawa et al., 2001). So, there is a growing demand for BR in America and European countries.

Black rice has been proven scientifically for its various pharmacological activities. Research has also confirmed the antioxidant activity, antitumor, anti-inflammatory, anti-proliferative, anticancer, anti-diabetes and hyperlipidemia, and anti-atherosclerosis activity of black rice, the cholesterol and lipid metabolism, and the tyrosinase inhibitor activity (Prasad et al., 2019; Liu et al. 2020). Moreover, the US Food and Drug Administration has recognized black rice as a healthy whole grain capable of decreasing the risk of certain diseases. However, it is not accepted by some people, as it is difficult to cook and may be due to its distinct off-taste, dark appearance and hard-cooked rice texture (Kushwaha, 2016).

Several applications of BR in dairy industries including Gabr et al. (2016) found that fortification of buffaloe`s milk with BR as a natural antioxidant source increased the antioxidant activity of soft cheese with decreasing the syneresis rate. Sensory evaluation showed that adding 5% black rice powder to buffaloe`s milk gained higher acceptability. Nurliyani et al. (2015) studied the effect of adding BR extract and goat milk on kefir characteristics and its improvement of pancreatic ß-cell in diabetic rats. It was found that 2 ml dose of kefir contained goat milk and BR extract was effective to achieve similar healing effect to glibenclamide as antidiabetic agent. Recently, black rice bran was used as a dark purple color of anthocyanins additive in yoghurt (Nontasan et al., 2012). Moreover, Mongkontanawat (2016) processed a probiotic germinated native black rice milk mixture fermented by lactic acid bacteria (*Lactobacillus casei* TISTR 390). Recently, Liu et al. (2020) found that supplementation with whole grain black rice (WGBR) as a staple crop rich in bioactive compounds significantly reduced lipid accumulation and normalized the expression levels of hepatic and intestinal lipid metabolism-related proteins or genes in C57BL/6J mice. These findings supported that WGBR could prevent obesity-related dyslipidemia and hepatic steatosis.

It was supposed that the conditions of processed cheese production including heat treatment would intensively reduce the black rice rigidity and enhance its microbiological quality. In the present study, attempts were done for using black rice powder as a natural source of bioactive compounds and natural antioxidants in the manufacturing of PCSs and investigating its impact on the physicochemical, rheological properties, microbiological and sensory characteristics of processed cheese spreads.

Materials and Methods

Materials

Full ripened Cheddar cheese (65.2% total solids (TS), 38.2% fat/dry matter (F/DM) and Tallaga cheese (34.2% total solids, 16.9% F/DM) were obtained from the local market. Black Rice was obtained from Sakha Agricultural Research Station, Kafr El-Sheikh, Egypt. BR was milled using Cyclotec milling (Cyclotec ™ 1093, Foss, Sweden) and sieved using a 60-micrometer sieve. The obtained black rice powder (BRP) was used in the processed cheese mix. Joha 89 emulsifying salt (BK Giulini chemie GMBH, Landenburg, Germany) was obtained from the local market.

Methods

Manufacture of processed cheese spreads

Processed cheese products were made mainly from blends containing imported Cheddar with local soft cheese type (Tallaga cheese) with skim milk powder (97% TS product of Dairy America™) and milk protein concentrate to control active milk protein content. Amounts of different ingredients for making of full-fat PCS treatments were calculated in order to fulfill the Egyptian standard specification of the final product, i.e dry matter (DM) should not less than 36% and fat on dry basis (F/DM) should be in the range of 35-65%. The required ingredients were mixed, placed in the processing kettle (model Stephens Universal machine, Switzerland) each of 2.5 kg capacity and then heated by direct steam up to 93±1ºC with continuous mixing at 1400 rpm for 6 minutes. Thereafter, the heating was stopped and the hot product was filled immediately into a wide open sterile screw capped glass cups (200 ml capacity). PCSs were allowed to cool to the room temperature and thereafter stored under refrigeration (4±1°C) until analyzed when fresh, and after 1, 2 and 3 months for some parameters during storage at refrigerator (4±1°C). The different formulations of PCSs are presented in Table (1). There are four treatments; the first one was prepared without using black rice powder (BRP) as a control. Other treatments including a gradual replacement of dairy ingredients (Cheddar, Tallaga cheeses, skim milk powder and milk protein concentrate) with 2, 4 and 6% BRP as T_1, T_2 and T_3 respectively.

Chemical analysis

The fat content of processed cheese samples was determined by Gerber's method and the protein content was determined by Kjeldahl method (AOAC, 1990). Similarly, total solids and the pH values were determined according to AOAC (1990).

Determination of total phenolic compounds and antioxidant scavenging activity

Five grams of sample were mixed with 50 ml of 50% ethanol and stirred at room temperature for 1 h and filtered through Whatman no. 1 filter paper. TPC was determined in the ethanolic extract as described by Singleton and Rossi (1965). Results were expressed as mg of gallic acid equivalents (GAE) / 100g of the sample. The antioxidant activity of the sample was evaluated by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay Cuendet and Potterat (1997) and Burits and Bucar (2000). The DPPH radical-scavenging activity was calculated using the following formula:

$$\text{DPPH radical – scavenging activity (\%)} = [(1 - A_1 / A_0) \times 100]$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

Microbiological analysis

The total viable bacterial count (TVBC) was determined as described by Marshall (1992). The mould and yeast counts were enumerated using potato dextrose agar (APHA, 1985) and plates were incubated at 25 ± 2º C for 4-5 days.

The color readings

The color reading values of different PCS treatments were measured using a light reflectance spectrophotometer (Mionolta, CR 300, Osaka, Japan). Measurements were recorded as L^* to express (lightness), $+a^*$ (redness) and $+b^*$ (yellowness) based on CIE (Commission Internationale de I'Eclairage) color coordinates.

TABLE 1. Formulation of different spreadable processed cheese for 100 kilogram mix

Ingredients	Amount/100 kg			
	Control (C)	T_1	T_2	T_3
Matured Cheddar cheese	22.0	20.0	18.0	16.0
Tallaga cheese	42.5	39.5	36.5	33.5
Butter	7.45	9.0	10.5	12.0
Skim milk powder	4.0	3.25	2.55	1.75
Milk protein concentrate	1.0	1.0	1.0	1.0
Emulsifying salts	3.0	3.0	3.0	3.0
Black rice powder	0	2.06	4.12	6.18
Water	20.05	22.19	24.33	26.57

Control (C): without addition of BRP, T_1: PCS enriched with 2% BRP, T_2: PCS enriched with 4% BRP, T_3: PCS enriched with 6% BRP.

Rheological characteristics of processed cheese samples

All treated samples were subjected to Texture Profile Analysis (TPA) under deformation force 25% of the sample. After manufacturing, the sample of PCS was filled up to 3.5 cm height of sample container (50 ml capacity, 5.5 cm height, 4 cm internal diameter) made of high density polyethylene and evaluated at 20°C for its textural attributes.

The organoleptic properties of processed cheese spreads

PCS samples were sensory evaluated by staff members and others at the Dairy dept., Faculty of Agriculture, Suez Canal University. The score card of PCSs was designed as the reported score card suggested by Meyer (1973) as follows: processed cheese appearance (20 points), body and texture (40 points) and flavour (40 points), which give total acceptability score of (100 points).

Statistical analysis

All measurements were done in triplicates, and analysis of variance was conducted by the procedure of General Linear Model (GLM) according to Snedecor and Cochran (1980) using Costat under windows software version 6.311 and least significant difference (LSD) test were employed to determine significant difference at $p<0.01$.

Results and Discussion

Proximate analysis of processed cheese spreads

Table 2 shows that the processed cheese spreads made from different replacements had no significant differences in dry matter or fat content ($p>0.01$). Fat/dry matter (Fat/DM, %) was affected by substituting dairy-based raw materials such as Cheddar, Tallaga cheeses and skim milk powder with gradual ratios of BRP. As seen in Table 1 and 2) different formulations were designed to make full fat spreadable processed cheese to meet the Egyptian regulation standard. In addition, using of BRP instead of natural cheeses and skim milk powder in PCS significantly decreased the protein content ($p<0.01$). This may be due to the higher carbohydrates content of BRP used instead of different dairy ingredients. Adding of BRP in different processed cheese treatments increased the pH values of resultant treatments. This may be attributed to the presence of alkaline salts in RBP. Similar finding was found by using black rice powder in kefir production by Nurliyani et al. (2015).

Total phenolic compounds and antioxidant scavenging activity

Changes in TPC and AA of different PCS treatments during three months of cold storage periods are presented in Fig. (1). It was shown that adding different ratios of BRP in PCS (T_1, T_2 and T_3) increased the TPC and AA compared to the control sample as fresh and throughout the cold storage period. The higher TPC of treated cheeses samples may be related to higher TPC content of BRP compared the control sample. While, the higher AA of treated cheeses could be attributed to the BRP content of phenolic compounds, anthocyanins and flavonoids (Pedro et al. 2016). Phenolic compounds may exert their antioxidant activity through a direct scavenge some reactive species or may bind pro-oxidant metals, such as iron, preventing the formation of free radicals (Walter and Marchesan, 2011). In addition, TPC and AA values tend to decrease gradually with extending the storage period of PCS because of possible oxidation process during cold storage period. Similar trend was reported by Lee et al. (2016) for Cheddar cheese during cold storage.

TABLE 2. Proximate composition of different processed cheese spread as affected by using substantial percentage of black rice powder*(average of triplicates).

Constituents	C	T_1	T_2	T_3
Dry Matter (DM)%	43.19±0.21^a	43.16±0.19^a	43.22±0.23^a	43.20±0.16^a
Fat (%)	21.62±0.1^a	21.61±0.1^a	21.65±0.1^a	21.64±0.1^a
Fat/DM (%)	50.06±0.02^a	50.08±0.02^a	50.09±0.02^a	50.09±0.02^a
Protein (%)	12.07±0.04^a	11.19±0.05^b	10.32±0.05^c	9.42±0.10^d
pH	5.56±0.03^d	5.60±0.02^c	5.65±0.03^b	5.71±0.02^a

* Mean ± Standard deviation.

Means with the same row with different superscript are significantly different ($p<0.01$).

T1: PCS enriched with 2% BRP, T2: PCS enriched with 4% BRP, T3: PCS enriched with 6% BRP.

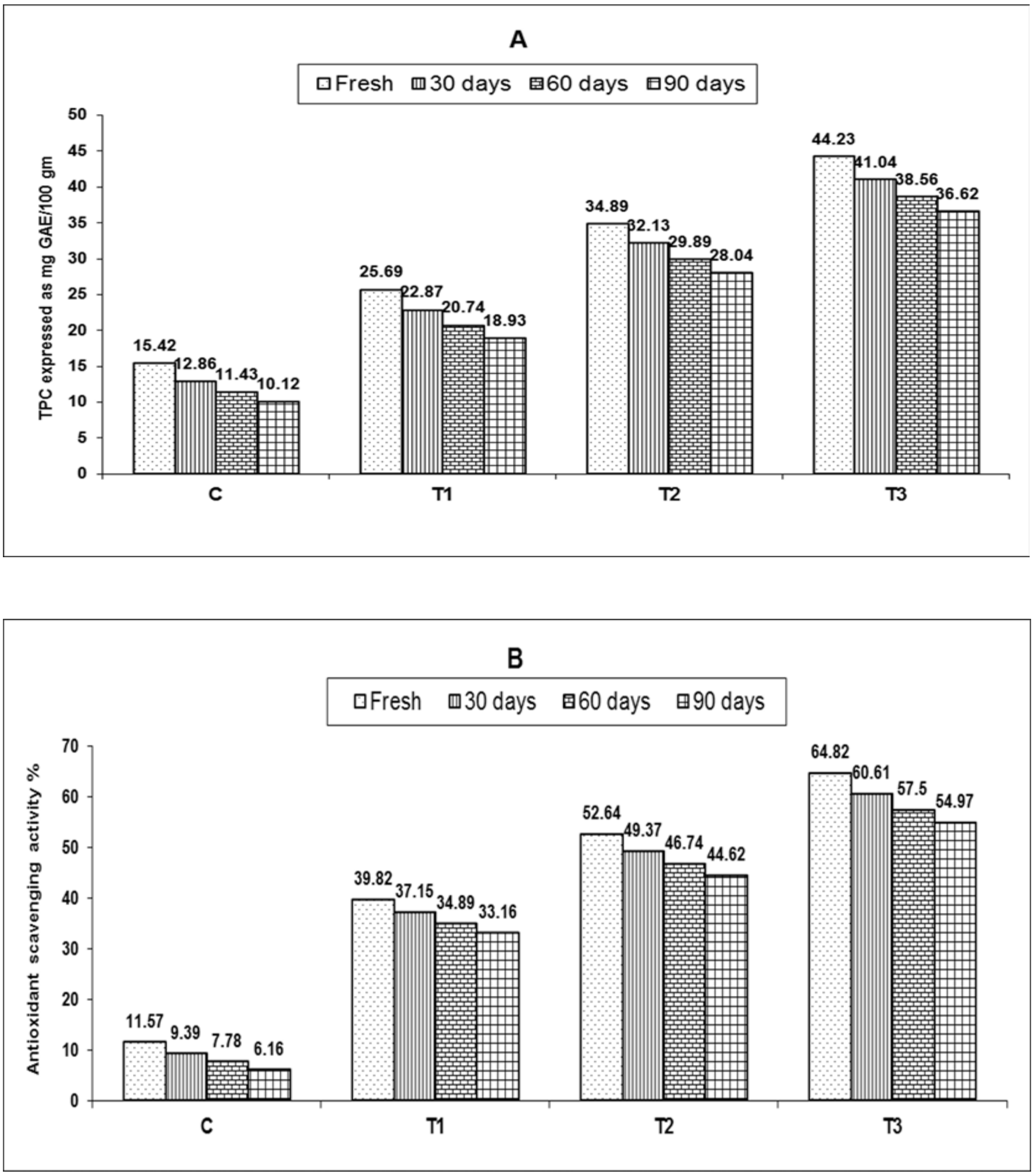

Fig. 1. Changes in total phenolic compounds expressed as mg gallic acid equivalent/100 g (A) and antioxidant scavenging activity % (B) of processed cheese spreads as affected by substantial amounts of black rice powder.

The rheological characteristics of different processed cheese treatments

Table 3 illustrates the rheological characteristics of different PCSs as affected by using substantial ratios of BRP. In addition of BRP increased all the rheological characteristics significantly ($P<0.01$) except springiness of processes cheese samples. These increases were parallel to the used ratio of BRP. This may be due to the presence of fiber and high carbohydrate content in BRP that may bind high percentage of the moisture in PCS causing an increase in the cheese hardness. The springiness had an opposite trend of different rheological parameters. This may be due to that harder cheese tended to have lower springiness values than the softer one. Similar findings were reported by Tawfek (2018) who observed that Hardness, cohesiveness, gumminess and chewiness exhibited proportionally higher values, while, springiness property behaved opposite trends as the black rice flour was used instead of cheese-base in spread type processed cheese recipes.

Microbiological quality and color reading

The inclusion of black rice powder in different concentration in the PCSs mix had no noticeable effect on the microbiological quality of the product (p>0.01, Table 4). Thus, small significant differences were found in the total viable count in both fresh and cold stored samples (after 3 months). However, moulds and yeasts were reported to be absent in market processed cheese, this result is in agreement with those obtained by Abd Alla et al. (1996) and Elshibiny et al. (2013). Total viable counts of different treatments were in the range as reported by Abd Alla et al. (1996) namely; 42-87x 10^2 cfu/g of PCS market. The presence of moulds and yeasts in control and BRP containing PCSs after 3 months of cold storage may be due to post contamination during the manual packaging of PCSs. From the foregoing results, it was clear that using BRP in PCS had no probable effect on total viable counts, moulds and yeasts content.

Color is a critical parameter to assess the quality of food products may be because its correlation with desirability and food quality (Nontasan et al., 2012). Instrumental measurements of color is based on the determination of three parameters i.e., *L* (whiteness), *a* (redness) and +b (yellowness). Table 4 presents the changes in color reading characteristics of different PCS treatments as affected by substantial ratios of BRP instead of dairy-based raw materials. It was found that replacing dairy-based raw materials (Cheddar cheese, Tallaga cheese and skim milk powder) with gradual ratios of the BR in PCS led to an increase in L^* and a^* values but a decrease in b^* value was noticed which can be attributed to the purple color of BR.

Sensory evaluation of different PCS treatments

Table 5 shows the sensory evaluation of different PCS as affected by using different BRP ratios. Results showed that adding 2 % BRP (T_1) had no significant (P<0.01) effect on total acceptability than that of the control sample. While using the higher ratios of BRP in PCS (T_2 and T_3) led to a gradual decrease in all the sensory characteristics. Despite of PCS with 4-6 % BRP tended to have higher rheological characteristics (hardness, fracture stress, Gumminess and chewiness); so the sensory scores were low compared with the control and T_1 sample. Similar trend was reported by Tawfek (2018). This was attributed to the presence of fiber and high carbohydrate content which had a negative effect on the spreadability of the final product. Furthermore, concentrations >2% BRP in PCS caused a decrease in the gain flavour scores.

TABLE 3. Rheological characteristics of different processed cheese samples as affected by using substantial percentage of black rice powder*(average of triplicates).

Parameter	C	T_1	T_2	T_3
Hardness (N)	1.82±0.10^d	2.05±0.10^c	2.31±0.13^b	2.52±0.11^a
Fracture stress (N)	1.63±0.06^d	1.85±0.07^c	2.18±0.06^b	2.56±0.08^a
Springiness (mm)	7.96±0.02^a	7.89±0.03^b	7.75±0.02^c	7.66±0.02^d
Gumminess (N)	1.53+0.03^d	1.64±0.04^c	1.78±0.04^b	1.86±0.03^a
Chewiness (mJ)	12.17±0.19^d	12.94±0.22^c	13.78±0.26^b	14.28±0.25^a

* See legend to Table 2 for more details.

TABLE 4. Microbiological quality* and color reading characteristics of processed cheese spreads containing different percentages of black rice powder (Average of triplicates)

Counts (10^2 Cfu /gm)	C	T_1	T_2	T_3
Viable total count fresh	53.3±0.5^b	56.2±0.6^a	54.1±0.6^b	57±0.6^a
Viable total count /3 months	73.9±0.7^b	75.3±0.6^a	74.1±0.7^b	76±0.7^a
Moulds and yeasts fresh	ND	ND	ND	ND
Moulds and yeasts /3 months	2.11±0.1^a	2.19±0.1^a	1.95±0.1^b	2.20±0.1^a
Color readings				
L^*	70.1±0.3^d	71.7±0.4^c	73.1±0.4^b	76.6±0.5^a
a^*	3.5±0.2^d	5.8±0.2^c	6.9±0.3^b	8.4±0.4^a
b^*	18.7±0.3^a	14.3±0.2^b	12.3±0.2^c	11.6±0.2^d

TABLE 5. Sensory properties of different spreadable processed cheese treatments (Average of triplicates).

Parameter		C	T_1	T_2	T_3
Fresh	Appearance (20)	19	19	18.5	18
	Body & Texture (40)	38	38	37	36
	Flavour (40)	39	38	37	36
	Total acceptability (100)	96±1.32[a]	95±1.30[a]	92.5±1.20[b]	90±0.97[c]
After 3 months of storage	Appearance (20)	18	18	17	16
	Body & Texture (40)	37	37	36	35
	Flavour (40)	37	36	35	34
	Total acceptability (100)	92±1.28[a]	91±1.42[a]	88±0.88[b]	85±0.92[c]

* Statistical analysis was carried out only for total acceptability scores of different ice cream treatments.
* See legend to Table 2 for more details.

Conclusion

Depending on the higher nutritive value and bioactive compounds including total phenolic compounds, anthocyanins and antioxidant scavenging activity with attractive purple color making black rice as a valuable component in dairy industries especially in spreadable processed cheese. The gradual replacement of cheese base with BRP in processed cheese spreads was associated with the proportional increment in the phenolic compounds (25.69, 34.89 and 44.23mg100/gm) as the control (15.42mg100/gm) and antioxidant scavenging activity (39.82, 52.64 and 64.82%) as the control (11.57%). It is recommended to use 2% black rice powder into processed cheese formula to improve the nutritional, rheological characteristics and total antioxidant scavenging activity of the resultant spreadable processed cheese with good total acceptability scores.

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الخصائص الوظيفية والجودة التغذوية لمفرود الجبن المطبوخ المدعم بمسحوق الأرز الأسود

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يحتوي الأرز الأسود علي العديد من المكونات الحيوية مثل البروتين، الالياف، الكربوهيدرات والعناصر المعدنية وكذلك يتميز بلونه الأرجواني الجذاب مما يجعله مكون غذائي ذو قيمة عالية يمكن إستخدامه في صناعة منتجات الألبان. تمت دراسة إمكانية الإستبدال الجزئي للمكونات اللبنية بنسبة ٢ - ٦٪ (٢،٠٦ ، ٤،١٢ و ٦،١٨ كجم) من مسحوق الأرز الأسود لتصنيع مفرود الجبن المطبوخ الوظيفية. تم تقدير التركيب الكيمياوي، الميكروبيولوجي، الخصائص الريولوجية، المركبات الفينولية، النشاط المضاد للأكسدة والتقييم الحسي لمفرود الجبن المطبوخ. أظهرت النتائج عدم وجود إختلافات معنوية في محتوي المادة الصلبة ونسبة الدهن لعينات الجبن المطبوخ مقارنة بالكنترول ومع ذلك إنخفضت نسبة البروتين بشكل معنوي نتيجة إضافة مسحوق الأرز الأسود. أدى تدعيم الجبن المطبوخ بنسب مختلفة من مسحوق الأرز الأسود إلى زيادة ملحوظة في محتوي المركبات الفينولية والمواد المضادة للأكسدة سواء في العينات الطازجة أو أثناء التخزين المبرد. علاوة على ذلك ، تأثرت الخصائص الريولوجية للجبن المطبوخ نتيجة التدعيم بالأرز الأسود. لم تؤدي إضافة مسحوق الأرز الأسود بتركيزات مختلفة إلى تأثيرات ملحوظة علي الجودة الميكروبيولوجية للجبن المطبوخ. أظهرت نتائج التقييم الحسي أن تدعيم مفرود الجبن المطبوخ بمسحوق الأرز الأسود بنسبة ٢٪ هي المعاملة الأكثر قبولاً مقارنةً بالكنترول. ولذلك يتضح إمكانية إستخدام مسحوق الأرز الأسود في إنتاج مفرود الجبن المطبوخ لإكسابه خصائص وظيفية وتغذوية جيدة وخواص حسية مقبولة.