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STORAGE STABILITY OF BLACK WHEAT-SUPPLEMENTED FUNCTIONAL COOKIES IN DIFFERENT PACKAGING MATERIALS: A SENSORY, PHYSICAL, COLOUR AND MICROBIAL QUALITY EVALUATION

Anuprita A. Joshi[1*], Surendra K. Sadawarte[1], Preeti P. Thakur[2], Pramodini More[3], Rajesh B. Kshirsagar[4], Mansi Sonekar[5] and Shruti Nakhat[6]

[1,2,4,5,6]College of Food Technology, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani-431401, Maharashtra, India
[3]College of Agricultural Engineering, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani-431401, Maharashtra, India
***Corresponding author**: Anuprita A. Joshi, E-mail: joshianuprita.cft@gmail.com

ABSTRACT
Black wheat is a nutrient-dense cereal valued for its anthocyanin, phytochemicals, dense amino acid profile and its incorporation into cookies offers a route to functional, nutraceutical-rich products. This study evaluated wheat flour cookies supplemented with black wheat flour at graded levels to identify a blend that combines acceptable sensory quality with improved physical, texture and microbial quality with stable storage quality. The incorporation of Black wheat flour in increased the weight of cookies and thickness and the diameter and spread ratio of the cookies was decreased progressively from with the increasing level of Black wheat flour. There was progressive increase in hardness of cookies with addition of Black wheat flour in composite cookies. The hardness increased from 8.123 Kg to 12.494 Kg. Black wheat supplemented cookies could be stored up to 180 days at ambient conditions by using aluminium foil as a suitable packaging material.

KEYWORDS: Black wheat, cookies, spread ratio, microbial quality, shelf life

INTRODUCTION
Wheat is one of the major cultivated crop around the globe. This grass (*gramineae*) family cereal plays a pivotal role in the human diet, as it is taken as the staple food.
A new wheat variety called black-grained wheat (BGW) has been produced in China over the past 20 years from a blue and purple line that already existed (Sun SC *et al* 1996, Sun SC *et al* 1999). In India black wheat variety was first prepared after 7 long years of work and dedication of Dr. Monika Garg at National Agri-Food Biotechnology Institute (NABI), Mohali. They crossed the Japanese variety (EC866732) with the high yielding cultivar of wheat PBW621. The variety was named as 'NABI MG'. Black wheat is a type of pigmented wheat variety which shows the dark colour on its outer cover (pericarp). Color is due the presence of anthocyanins as reported by Monika Garg et al (2017). This black colored wheat is now making headlines for its nutritive value. Different studies proves that the health benefits of this is way much better than the conventional yellow wheat variety in aspect of protein content, essential amino acids availability gluten index of its flour etc. Mallick and Sau (2021.) In view of the nutritional and nutraceutical profile black wheat, the present research work is planned to prepare and evaluate the physical and storage quality of black wheat incorporated cookies and to utilize black wheat for preparation of nutritious commercial value-added food products rich in nutraceuticals which also provides health benefits to the consumers.

MATERIALS AND METHODS
Black wheat grains were obtained from local area of Punjab state and traditional wheat variety Netravati as a control sample was procured from Seed Research Centre, VNMKV, Parbhani and for analysis and for preparation of cookies raw materials were procured from local market Parbhani.

Cookie formulation and preparation
All the cookies with different concentration of black wheat flour incorporation were prepared by AACC method (10-50D) with slight modifications with the same method as mixing ingredients, creaming, kneading, sheeting, cutting and baking in an preheated oven at 170°C for 15 min at College of Food Technology, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India.

Physical analysis of Black wheat supplemented cookies
Colour of wheat grains was measured by using Hunter Lab Colorimeter (Model No. Colour Flex EZ) in the Department of Horticulture, College of Horticulture, VNMKV, Parbhani. Hunter lab colorimeter measures the color in terms of the value L* (0=black, 100=white), a* (+value=red, -value=green) and b* (+value=yellow, -value=blue) with a standard white tile/board for setting the instrument with illuminant (Rajiv et al., 2015).
Black wheat supplemented cookies were analyzed for physical quality attributes. Weight was determined by direct weighing using electronic balance of 0.01g sensitivity. Diameter, thickness, spread factor were

determined following standard methods of methods of AACC (2000). Top grain development was visually assessed as a function of no. of cracks formed over the surface of the cookies.

Evaluation of textural qualities of Black wheat supplemented cookies

Textural qualities of Black wheat supplemented cookies was determined by adopting the method of Kang et al., (2017) using Stable Micro System TA-XT2 plus Texture Analyzer was used for texture profile analysis (TPA).Hardness of the baked cookies was measured in a compression mode with a sharp blade-cutting probe. Pre-test, test, and post-test speeds were 1.5, 2, and 10 mm/s, respectively. Hardness, a maximum peak force, was measured with more than six cookies for each sample. The peak force to snap the cookies was reported as fracture force in N.

Microbial analysis and Storage studies of prepared Black wheat supplemented cookies

Microbial quality of Black wheat supplemented cookies was determined by adopting the method of Pakhare et al., (2016) by determining total plate count (TPC) and yeast and mold count. The Black wheat supplemented cookies were packed in Low Density Polyethylene (LDPE) pouches, Polyethylene Terephthalate (PET) jar and aluminum foil. The sensory quality of them was evaluated at an interval of 60 days for a period of 6 months.

RESULTS AND DISCUSSION

Table 1 : Effect of different levels of Black wheat flour incorporation on the color value of Black wheat supplemented cookies

Sample code	Colour value		
	L^*	a^*	b^*
Control(N)	**63.54**	**8.80**	**28.74**
BC_1	59.49	8.15	28.23
BC_2	57.43	8.19	27.02
BC_3	**55.30**	**10.10**	**24.97**
BC_4	54.46	10.57	24.88
BC_5	52.74	10.67	23.23
SE±	**1.130**	**0.609**	**0.282**
CD at 5%	**3.522**	**1.898**	**0.879**

Black wheat flour incorporation in cookies preparation had a statistically significant ($p<0.05$) effect on L*, a* and b* values of cookies. The Accepted Black wheat supplemented cookies had L*=55.30, a*=10.10 and b*= 24.97 .The raw BC_1 sample showed highest L* value (59.49) L* value for Black wheat supplemented cookies which was decreased gradually from (59.49 to 52.74) with the increase in level of incorporation of Black wheat flour.

Table 2: Effect of different levels of Black wheat flour incorporation on physical analysis and texture profile of black wheat supplemented cookies

Sample no.	Physical Parameters						Texture profile
	Unit weight(g)	Diameter (cm)	Thickness (cm)	Spread ratio	Spread factor	Top grain	Hardness (kg)
Control	**16.32**	**5.75**	**1.25**	**4.6**	**46**	Rare	**7.121**
BC_1	16.48	5.77	1.10	5.24	52.42	Moderate	8.123
BC_2	16.72	5.58	1.17	4.77	47.72	Moderate	8.366
BC_3	**17.14**	**5.54**	**1.25**	**4.43**	**44.33**	**Excellent**	**10.681**
BC_4	17.60	5.54	1.33	4.17	41.67	Negligible	11.241
BC_5	19.46	5.33	1.38	3.86	38.65	Negligible	12.494
SE±	**0.094**	**0.041**	**0.015**	**0.049**	**0.163**		**0.078**
CD at 5%	**0.293**	**0.128**	**0.048**	**0.151**	**0.509**		**0.244**

The increase in percentage of Black wheat flour progressively resulted an increase in thickness from 1.10 cm (BC_1) to 1.38cm (BC_5) and the diameter of the cookies was decreased progressively from 5.77 cm (BC_1) to 5.33cm (BC_5). The spread ratio of 10% Black wheat flour was highest 5.24 and it was gradually decreased with the increased level of black wheat flour. The sample BC_3 had spread factor 4.43 which was significantly lower as compared to control sample *i.e.*, 4.6. However top grain of Black wheat supplemented cookies up to 30 per cent incorporation of black wheat flour was found to be excellent.

The hardness increased from 8.123 Kg to 12.494 Kg. The highest value of hardness observed in BC_5 (12.494 Kg) and lowest value of hardness was noted for control sample made from Netravati wheat flour (7.121 Kg).

Table 3: Effect of different levels of Black wheat flour incorporation on the Texture Profile analysis of Black wheat supplemented cookies

Sample	Hardness (kg)
Control	**7.121**
BC_1	8.123
BC_2	8.366
BC_3	**10.681**
BC_4	11.241
BC_5	12.494
SE ±	**0.078**
CD at 5%	**0.244**

*Each value is average of three determinations

Table 3 revealed that there was progressive increase in hardness of cookies with addition of Black wheat flour in composite cookies. The hardness increased from 8.123 Kg to 12.494 Kg. The highest value of hardness observed in BC_5 (12.494 Kg) and lowest value of hardness was noted for control sample made from Netravati wheat flour (7.121 Kg). Increase in hardness of cookies may be due to replacement of wheat flour by Black wheat flour rich in protein and fibre. Similar results were found by Hoseney and Rogers (1994) as increase in the hardness of wheat protein made cookies due to interaction of protein and starch by hydrogen bonding. Similar results were endorsed by Soni *et al* (2018**)** as increased breaking strength of the cookies in proportion to the wheat flour because of higher protein content in treated flours than wheat flour.

The increased level of Black wheat flour in cookies imparts more hardness in almost all samples of Black wheat supplemented cookies, due increased level of protein content, however on the basis of sensory evaluation, sample BC_3 was liked and recorded higher score in all sensory attributes, which correlates with textural analysed hardness(10.681Kg).The results are in good agreement with the results reported by Kulkarni and Joshi (2013) and Arshad *et al*. (2012).

Table 4: Microbial analysis of selected Black wheat supplemented cookies

Storage period (In days)	Microbial quality (cfu/g)			
	TPC (log cfu/g)		Yeast and mold (log cfu/g)	
Sample	**BC_3**	**Control**	**BC_3**	**Control**
0	ND	ND	ND	ND
60	ND	1.2	ND	1
120	2.3	3.23	2.2	2.4
180	3.19	3.55	3.2	3.4

*Each value is average of three determinations; ND- Not detected

From table 4 it is noted that in selected Black wheat supplemented cookies on the basis of sensory evaluation nothing was noticed in TPC and yeast mold count up to 60days of storage however in control cookies from Netravati wheat flour 1.2log cfu/g in TPC noted at 60days of storage and yeast mold count there was 1log cfu/g observed. The total plate count of control cookies varied from 1.20 to 3.55 log cfu/g during 60 to 180 days of storage. The total plate count of Black wheat supplemented cookies (BC_3) ranged from 2.3 to 3.19 log cfu/g which was found to be overall less as compared control cookies sample. Similar trend followed for the yeast mold count and it was less in Black wheat supplemented cookies (BC_3) ranged from 2.2 to 3.20 log cfu/g for 120 to 180days of storage days and 1 to 3.4 log cfu/g for 60-180days storage period respectively. Total plate count increased significantly ($P \leq 0.05$) with the increase in period of storage. It was increased may be due to cross contamination while handling and storage. Agu and Ndidiamaka, (2014) reported that the growth observed could be due to post processing contamination. These results are similar with the results obtained by (Banusha & Vasantharuba 2014, Chopra *et al*., 2014, Peter-Ikechukwu *et al*., 2018; Goyat *et al*., 2018).

In conclusion Black wheat supplemented cookies with 30 per cent incorporation of germinated Black wheat flour could successfully be stored upto 180 days with acceptable microbial quality as per reported standards. The developed cookies therefore have the potential for commercialization. Different packaging materials need to be explored to retain the health benefits of such products.

Effect of packaging materials on sensory attributes of selected Black wheat supplemented cookies for different storage period

The selected Black wheat supplemented cookies containing 30 percent Black wheat flour *i.e.*, BC_3 sample were packed in LDPE pouch, PET jar and aluminium foil pouch and stored under ambient temperature for 180 days at Department of Food Engineering, College of Food Technology, V.N.M.K.V., Parbhani and sensory evaluation of product was conducted using semi trained panel members on 9 point hedonic rating. During storage, the samples were taken at different periods of time (60days of interval) to study their quality in terms of overall

acceptability. The results pertaining to storage study of Black wheat supplemented cookies in comparison with Netravati wheat cookies as control sample were summarized under following suitable heading.

Table 5: Effect of packaging materials on sensory attributes of selected Black wheat supplemented cookies in Low Density Poly Ethylene bags (LDPE) for different storage period

Parameters	Days	Samples		Mean	S.E +	CD at 5%
		C	BC_3			
Color	0	8.40	8.23	8.32	0.021	0.067
	60	8.36	8.16	8.26	0.017	0.059
	120	8.31	8.13	8.22	0.032	0.11
	180	8.20	8.01	8.11	0.040	0.14
Flavor	0	8.40	8.65	8.53	0.029	0.089
	60	7.61	8.58	8.10	0.037	0.12
	120	7.31	8.50	7.91	0.042	0.14
	180	6.20	7.70	6.95	0.084	0.29
Taste	0	8.60	8.65	8.63	0.149	0.46
	60	7.65	7.70	7.68	0.030	0.10
	120	7.43	7.52	7.48	0.055	0.19
	180	6.20	7.43	6.82	0.022	0.76
Texture	0	8.70	8.85	8.78	0.066	0.20
	60	8.61	8.74	8.68	0.022	0.63
	120	8.24	8.31	8.28	0.033	0.11
	180	7.60	7.65	7.63	0.038	0.13
Overall Acceptability	0	8.50	8.80	8.65	0.048	0.15
	60	7.78	8.18	7.98	0.027	0.093
	120	7.45	7.69	7.57	0.067	0.23
	180	7.43	7.46	7.45	0.050	0.17

*Each value is average of three determinations

Table 6: Effect of packaging materials on sensory attributes of selected Black wheat supplemented cookies in Polyethylene Terephthalate (PET) Jar for different storage period

Parameters	Days	Samples		Mean	S.E +	CD at 5%
		C	BC_3			
Color	0	8.40	8.23	8.32	0.149	0.46
	60	8.36	8.19	8.28	0.022	0.077
	120	8.32	8.16	8.24	0.010	0.034
	180	8.28	8.13	8.21	0.054	0.18
Flavor	0	8.40	8.65	8.53	0.037	0.12
	60	8.32	8.60	8.46	0.027	0.093
	120	8.28	8.50	8.39	0.034	0.11
	180	7.73	7.79	7.76	0.022	0.077
Taste	0	8.60	8.65	8.63	0.021	0.067
	60	7.70	7.79	7.75	0.065	0.025
	120	7.53	7.55	7.54	0.022	0.077
	180	6.28	7.45	6.87	0.057	0.19
Texture	0	8.70	8.85	8.78	0.048	0.15

	60	8.61	8.79	8.70	0.030	0.10
	120	8.24	8.35	8.30	0.053	0.18
	180	7.69	7.70	7.70	0.027	0.095
Overall Acceptability	0	8.50	8.80	8.65	0.066	0.20
	60	7.85	8.20	8.03	0.024	0.083
	120	7.50	8.28	7.89	0.010	0.034
	180	7.48	7.51	7.50	0.037	0.12

*Each value is average of three determinations

Table 7: Effect of packaging materials on sensory attributes of selected Black wheat supplemented cookies in Aluminum Foil for different storage period

Parameters	Days	Samples		Mean	S.E +	CD at 5%
		C	BC_3			
Color	0	8.40	8.23	8.32	0.014	0.46
	60	8.38	8.21	8.30	0.041	0.14
	120	8.32	8.17	8.25	0.036	0.12
	180	8.29	8.14	8.22	0.022	0.078
Flavor	0	8.4	8.65	8.53	0.029	0.089
	60	8.35	8.63	8.49	0.032	0.11
	120	8.30	8.55	8.43	0.030	0.10
	180	7.75	7.80	7.78	0.042	0.14
Taste	0	8.60	8.65	8.63	0.066	0.20
	60	7.88	8.00	7.94	0.038	0.13
	120	7.55	7.60	7.58	0.032	0.11
	180	7.50	7.55	7.53	0.051	0.17
Texture	0	8.70	8.85	8.78	0.048	0.15
	60	8.62	8.80	8.71	0.028	0.097
	120	8.26	8.50	8.38	0.067	0.23
	180	7.70	7.75	7.73	0.029	0.10
Overall Acceptability	0	8.50	8.80	8.65	0.021	0.067
	60	7.86	8.80	8.33	0.049	0.17
	120	7.76	8.36	8.06	0.025	0.086
	180	7.51	7.58	7.55	0.051	0.178

*Each value is average of three determinations

Color

It is evident from Table 5,6 and 7 that the selected Black wheat supplemented cookies sample BC_3 which had Black wheat flour incorporated at 30% with 70% control Netravati white wheat flour packed in polyethylene bag showed more changes in color and appearance during 180 days of storage as compared to aluminum foil and polyethylene terephthalate (PET) jar. The aluminum foil and pet jar stored samples showed non-significant changes in color as compared to polyethylene bag during 180 days of storage. The migration or absorption of moisture results in loss of colour during storage (Bender 1966). There is also natural tendency of colour fading with progressive storage which ultimately affects the appearance (Manley 2011).

The polyethylene bag is having low barrier to moisture as compared to aluminum foil and polyethylene terephthalate (PET) jar. Hence polyethylene packed samples showed more changes in color during 180 days of storage as compared to aluminum foil and polyethylene terephthalate (PET) jar. Similar results were obtained for cookies fortified with rectinyl acetate packed in bioriented poly propylene*i.e.*, decrease in color scores

during storage as reported by Mahmood *et al*. (2008). The decreasing trend in quality scores for appearance of cookies was due to the increase in moisture that has inverse correlation with appearance.

Flavor

Storage period had pronounced effect on the flavour of the cookies and greatly affected the sensory quality of both selected Black wheat supplemented cookies and the control cookies. Flavour score for all cookies was found to be progressively decreasing in LDPE throughout the storage as compared to aluminum foil and polyethylene terephthalate (PET) jar. The aluminum foil and polyethylene terephthalate (PET) jar packed cookies exhibited marginal decrease in flavor during 90 days storage. More pronounced effect was observed in control cookies followed by Black wheat supplemented cookies which might be due to oxidation of fats that further accelerated flavor deterioration.

Decrease in flavor was less in aluminum foil, it may be due to aluminum foil protects cookies from light, as light acts as a catalysts for oxidation and also because of its impervious nature to water vapors similar results found by Nagi *et al*. (2012). Better flavour retention in Black wheat supplemented cookies can be attributed to natural antioxidants present in the Black wheat flour and their strong antioxidant activity which might have delayed the onset of rancidity.

Taste

It can be observed from the results that selected Black wheat supplemented cookies packed in the LDPE pouch showed pronounced decrease in the score and cookies packed in aluminum foil and polyethylene terephthalate (PET) jar exhibited less decrease in taste score during 180 days of storage. Taste was found to be affected probably due to lipolytic changes; hastened by increase in moisture content of cookies(Wade *et al*; 1988).The fresh cookies at 0 days of storage scored maximum as compared to 60, 120, 180 days storage sample. With storage taste was progressively decreased.

Texture

It is clearly evident from the 5,6 and 7 that texture of selected Black wheat supplemented cookies progressively decreased during storage in all the packaging material. The table showed that cookies packed in aluminum foil and polyethylene terephthalate (PET) jar exhibited minimum changes in texture during 180 days of storage as compare to polyethylene bag.

The decrease in quality score of texture due to absorption of moisture that has negative effect on texture McWatters *et al* (2003). Aluminum foil and polyethylene terephthalate (PET) jar are good barrier to water vapour transmission rate. So the cookies packed in these materials exhibited lesser changes in texture of samples. The decrease in the texture scores of controls and selected Black wheat supplemented cookies was pronounced in LDPE pouches as compared to Aluminum foil and polyethylene terephthalate (PET) jar due to poor moisture barrier property of LDPE.

Overall acceptability

It is clearly evident from 5,6 and 7 that overall acceptability decreased progressively during storage in all the packaging material. Pronounced effect on overall acceptability of the selected Black wheat supplemented cookies and control cookies was found in LDPE pouches and it was observed that overall acceptability of the cookies stored in LDPE significantly decreased than in other packaging materials with the passage of time. It is evident that selected Black wheat supplemented cookies packed in aluminum foil and polyethylene terephthalate (PET) jar showed comparatively better overall acceptability during 180 days of storage as compare to LDPE. Among all packaging materials aluminum foil exhibited minimum decrease in overall acceptability and got highest scores as compare to both low density polyethylene (LDPE) and polyethylene terephthalate (PET) jar. Better overall acceptability in cookies packed in aluminum foil could be attributed to lesser deteriorative changes like development of rancidity and ingress of moisture.

The decrease in score for overall acceptability was probably due to increase in moisture during storage, proteolitic and lipolytic changes leading to decrease in quality score for colour, flavor, taste, texture and appearance of cookies. Similar decrease in scores for overall acceptability was observed **by** Pasha *et al*. (2002) and Mahmood *et al*. (2008) in cookies during storage intervals.

Thus, from the results obtained in the present investigation selected Black wheat supplemented cookies showed better overall acceptability in all packaging materials as compared to control cookies. The similar results for all sensory characteristics of different cookies packed in different packaging materials were obtained by Sahni and Shere(2017).All cookies stored in aluminum foil showed good score for overall acceptability and could be stored up to 180 days or even more at ambient conditions by using aluminum foil as suitable packaging material.

DISCUSSION

Physical properties of cookies are important from consumer point of view and also helps in evaluating the effect on baking due to replacement of Black wheat flour. The increased levels in thickness and decrease in diameter of Black wheat supplemented cookies might be due to replacement of Black wheat flour in increased proportion resulting in increase in percent protein content. A.A. Yousaf *et al*. (2012) concluded in their study as there was increase in protein content of cookies due to gram flour supplementation which in turn resulted in increased thickness and non-significant effect was observed in the width of the cookies. The changes in diameter and thickness were reflected in spread ratio of Black wheat supplemented cookies. According to Rababah *et al*. (2006), the spread ratio decreased when the protein content of cookies increased due to the use of more

gram flour. And this might be because biscuit dough made from high-protein flours has more hydrophilic sites available that compete for the limited free water (Khan *et al*. 1995). Hooda and Jood (2005) also reported that the spread factor of biscuits was decreased by increasing the supplementation levels of fenugreek flour in wheat flour.

Varying the incorporation level of Black wheat flour progressively increased hardness of cookies. Increase in hardness of cookies may be due to replacement of wheat flour by Black wheat flour rich in protein and fibre. Similar results were found by Hoseney and Rogers (1994) as increase in the hardness of wheat protein made cookies due to interaction of protein and starch by hydrogen bonding. Similar results were endorsed by Nihir Soni *et al* (2018) as increased breaking strength of the cookies in proportion to the wheat flour because of higher protein content in treated flours than wheat flour. The increased level of Black wheat flour in cookies imparts more hardness in almost all samples of Black wheat supplemented cookies,due increased level of protein content, however on the basis of sensory evaluation,sample BC_3 was liked and recorded higher score in all sensory attributes,which corelates with textural anlysed hardness(10.681Kg).The results are in good agreement with the results reported by Kulkarni and Joshi (2013) and Arshad *et al*. (2012).

The results for colour analysis were found more or less similar with Klunklin and Savage (2018) who reported the decrease in L* value due to substituting purple rice flour for wheat flour in increasing order in biscuits. In case of all cookie's treatments of increasing order of Black wheat flour addition, it indicated a color increase in the order of control < BC_1 < BC_2 < BC_3< BC_4< BC_5; same as visually observed.

CONCLUSION

The consumption of foods based on Black wheat would be important step towards alleviating protein malnutrition. The present research study recommends the prospect of more aggressive introduction and utilization of Black wheat in the food sector. It also implies that it may be worthwhile for industry to take up the production of Black wheat value added products and increasing cultivation by farmers. Such promotion of Black wheat value added products into diet in India could go a long way towards not only alleviating micronutrient deficiencies but also towards the development of functional, nutraceutical foods for preventing or curing of many lifestyles related diseases.

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