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BIOACTIVE ENRICHED CAKE SUPPLEMENTED WITH FRUIT SEGMENTS OF POMELO

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ABSTRACT

The aim of the present study was to develop a cake product using the fruit segments of *Citrus maxima* enriched with bioactives for nutritional benefits. The pomelo fruit segments was supplemented in fresh and dried form at various concentrations, The cakes were analyzed for physiochemical property the texture value decreased from 973 to 875 g force for fresh segments and 934 to 811 g force for dry segments. Despite of the cooking process In spite of the cooking process, bioactive compounds such as phenolics, flavonoids, and carotenoids are better preserved in cooked cakes, . Cakes supplemented with 30% fresh and 5% dry fruit segments was found to be sensorial acceptable. The sensorial preferred concentration was analyzed for proximate composition in which supplemented cake showed significant difference for total fat, protein and ash content as compared with control cake. Thus, the supplementation of pomelo fruit segments can be utilized as an ingredient for use in developing various products that aids in promoting health benefits.

Keywords: *Citrus maxima*, Cake, Sensory, Bioactives, Proximate

INTRODUCTION

Nowadays there is a growing interest by consumers, medical community and the public health organizations to utilize dietary means to enhance the health and wellness of the human beings. Beyond basic nutrition, the increasing awareness towards the reduction of disease and health benefits has led to significant growth in the area of functional foods and nutraceutical **(Zhao & Xie, 2004)**. Besides the effects of adequate nutrition, the functional food aid in reducing the risk of disease by providing health benefits to the body **(Vassallo *et al.* 2008)**. The phytochemical constituents in the functional foods plays a vital role in enhancing its therapeutic potential against various disorders **(Jenkins *et al.* 2008)**. The American Dietetic Association reported the scientific confirmation for the role of functional foods in the treatment and prevention against cancer, diabetes, cardiovascular disease and hypertension and further it has reduced the health cost which provides a provision for financial development in rural communities **(Bloch & Thomson, 1995)**. In earlier days, fortification with vitamin C, vitamin E, minerals and calcium were used for the development of functional foods **(Siró, 2008)**. Later, the functional foods with fortified micronutrients and bioactive components were produced in order to decrease the disease state and to promote the health benefits **(Siró, 2008)**. Hence initiative has been taken by the industries to develop a food product that aids in treating aginst various health disorders **(Siró, 2008)**. *Citrus maxima* are citrus fruit which is reported for its medicinal value **(Reshmi *et al.* 2017)**. As these functional food products have been mostly initiated in the bakery and dairy and industries this particular study has been undertaken to develop a functional food cake enriched with bioactive component from *Citrus maxima* fruit.

MATERIALS AND METHODS

Commercial whole wheat flour (Aashirvaad Superior Atta, India) having 7.5% moisture **(Reshmi *et al.* 2017)**, 10.1% protein and 0.45% ash was procured from local market.

Preparation of Pomelo fruit Segments

This citrus fruit, pomelo was obtained from a local market in Mysore, Karnataka, India in the month of February. To obtain dry fruit segments, the fresh segments were dried in a hot air oven for six hours at 53*2°C, then stored in the refrigerator until use for preparing the cake **(Reshmi *et al.* 2017)**.

Baking of cake

Cakes were prepared from whole wheat flour (control) as reported by **Sudha *et al.* (2007)** and **Ayadi *et al.* (2009).** The composition was 100g flour, 100g sugar, 120g egg, 100g of margarine, 0.5g baking powder and 1.5g salt. The cake batter was made using the flour batter method, in which flour, salt, shortening, and baking powder were creamed with eggs and sugar to obtain semi-firm foam; the flour batter method was used to prepare the cake batter. The flour –fat cream was gradually added to sugar-egg foam and mixed to obtain a uniform batter. The batter (450g) was poured into a wooden pan and baked at 160 ^{0}C for 1h (APV Rotell rotary oven, Australia). Fresh pomelo fruit segments at 0, 10, 20 and 30% and dried pomelo segments at 0, 2.5, 5 and 7.5% were mixed with batter at the final stage of mixing and cakes were prepared. As soon as they were baked, they were cooled to room temperature for 2 hours. They were then packed in polypropylene pouches until further analysis.

Physical characteristics of cakes

According to the standard method of AACC (2000) with slight modifications, the weight and volume of cakes were measured using the rape seed displacement method. Cake texture was determined objectively with a food texturometer (TAHDi, UK) by measuring the compression force with an aluminum cylinder probe (35mm diameter) and a test speed of 1.67mms-1 at a pre-test speed of 2.0mms-1. (**Sudha *et al.* 2007**). The force required to compress 25% of a cube of one-inch cake was measured. Using Hunter Lab's Color Measuring System (Labscan XE, Hunter Associates Laboratory Inc, Reston, USA) and a reflectance attachment of illuminant G, an objective evaluation was conducted to measure the color of the cake crumb versus a standard white board made of barium sulphate. Measurement was made by placing a cake piece placed on a sample holder and the reflectance measured from its surface **(Reshmi et al., 2017b)**.

Sensory analysis and Proximate composition of cakes

Panelists in the age group of 25 to 50 who were well versed with the quality parameters and the sensory evaluation of cakes was carried out using nine hedonic quality dimensions, including color, shape, crumb colour, texture, taste, and overall quality **(Sudha et al., 2017)**. Samples were coded and served to the panelists one by one for assessing the attributes. The proximate composition of the product such as moisture, crude protein, fat and ash contents was determined by the methods of AOAC (2005).

Preparation of cake sample for estimations

The samples were dried using hot air oven for 6 hours at 53*2°C. After cooling at room temperature, the homogenized samples were placed in polypropylene bags and stored at 4°C, the samples were retrieved from the refrigerator and used for further analysis **(Reshmi *et al.* 2017)**.

Total phenolic content

Using a Folin-Ciocalteu colorimetric assay (Ainsworth & Gillespie, 2007), the total phenolic content was determined by incubating 1 ml of the extract with Folin-Ciocalteu reagent followed by 2 ml of 10% sodium carbonate solution.for 1h at room temperature. **(Reshmi *et al.*, 2020)**. Total phenolic content of the mixture was expressed in mg gallic acid equivalents/g extract by reading its absorbance at 765 nm.

Flavonoids

According to Chai & Wong, 2012, the flavonoid content of the extract (0.9 ml) was determined by adding 10% alumimium chloride and 5% sodium nitrite solution to it. After incubation (6 min), sodium hydroxide (1M) was added and left to stand for 15 minutes at room temperature.. An absorbance measurement was performed at 510 nm to determine the total flavonoid content in mg catechin equivalents per gram of extract **(Reshmi *et al.*, 2020)**.

Carotenoids

Samples (1g) were extracted three times with 25 mL of acetone in the dark to prevent oxidation of the carotenoid content as described by **Reshmi *et al.*, (2020)**. In the separating funnel, 10 mL of the extracted product was mixed with 40 mL of petroleum ether. The upper portion of the petroleum ether containing carotenoids was separated. A volume of petroleum ether was used to make up the sample, and it was measured at 450 nm. The total carotenoid content was calculated using the formula below **Carvalho *et al.* (2012)**

$$\text{Carotenoids content}\left(\frac{\mu g}{g}\right)=\frac{A \times V\,(mL) \times 10^4}{A_{1\%}^{1cm}\ x\ P(g)}$$

where A = Absorbance; V = Total extract volume; P = sample weight; $A_{1\%}^{1cm}$=2592 (β-carotene Extinction Coefficient in petroleum ether).

***Invitro* digestion method**

The experimental was organized as described by **Xavier *et al.* (2014)** with slight modifications. The prepared cake which is found to be sensorially acceptable with higher retention of bioactive compounds was chosen for this experiment to determine the bioaccessibility of compounds by *invitro* digestion method. To the sample 0.2 g of pepsin was mixed with 0.1M HCl (pH 2.2) and incubated at 37ºC for 2h. The partially digested sample was mixed with bile extract (0.3%) and the pH was adjusted to 7.0 and incubated at 37ºC for 30mins. Further the incubated mixture was cooled and 5 mL of pancreatin (4 g/L) and bile (25 g/L) mixture was added and incubated for 2 h at 37ºC (Shao et al., 2015). The digested sample was then centrifuged and the supernatant was collected and accessed for the measurement of phenolics, flavonoid and carotenoid content to determine the bioaccessibility efficiency. The percentage of bioaccessibility was calaculated using the following formula:

$$Bioaccessibility\ (\%)=\frac{BC_{digested}}{BC_{non-digested}}$$

where $BC_{digested}$ concentration of bioactive compound in the digested cake sample (dialyzed fraction for, phenolic, flavonoids and carotenoids). $BC_{non-digested}$ was the concentration of these compounds in the non-digested cake samples.

RESULTS AND DISCUSSION

Quality characteristics of pomelo incorporated cakes

Table 1 shows characteristics of the cake based on the addition of pomelo fruit segments. The cake weight increased for added segments from 379.70 to 396.82 g, even though the difference between control and supplement is not very significant. The supplementation of pomelo fruit segments caused increase in cake volume from 1090 to 1189 cc for 10-30% and it decreased from 950-839 cc for 2.5-7.5% respectively. Similarly, in the case of specific volume, there was an increase from 2.84 to 3.05cc/g for fresh segments whereas for dry segment supplementation it decreased (2.46-2.11cc/g). In their previous study, **Singh *et al.* (2013)** reported that cake volume and specific volume significantly increased with increasing fiber content. **Qureshi *et al.* (2017)** reported that, fiber hinders the release of water and reduces the aeration of the cake batter, thereby causing the volume of the cake to decrease. According to the above statements, the inclusion of more dried fruit segments in the cake decreased its volume as the fibre content increased. The batter density increased from 0.40 to 0.47 g/cc for dry segment supplemented cake whereas for fresh segment supplementation it decreased (0.35-0.32 g/cc) as compared against control (0.43). **Lu *et al.* (2010)** stated that, fibers and carbohydrates in the supplementation have the potential to aerate the batter more effectively, thereby increasing the batter density by disrupting its structure. With increasing levels of fortification, the pomelo fruit segments have naturally increased the batter density due to their fiber and bioactive constituents **(Reshmi *et al.* 2017)**. Hence the differences observed in the formulation might be due to the effect of ingredients on this internal structure. The texture value decreased from 1093 to 811 g force which denotes the increase in the softness of the cake with increase in level of fruit segments. According to **Seker (2005)**, the change in texture is caused by the interaction between protein and starch, which prevents the chain of amylopectin from expanding and prevents water vapor from being released during baking. Lightness, redness, and yellowness were all expressed as Hunter L*, a*, and b* values, respectively (Table 1; Figure 1). The increase in the level of pomelo fruit segments decreased the L* value (58.24-54.85) while the a* value (4.37-5.61) increased indicating the increase in darkness and redness due to the substitution of fruit segments. The yellowness (b* value) varied with the control (18.53-19.50) but there is no significant difference between 2.5-7.5% substituted cake. Raw materials used in the formulation play a huge role in determining the color of the crumb. As the internal temperature of the cake fails to reach 100°C, maillard or caramelization reactions will not occur. **(De La Hera *et al.* 2012; Gómez *et al.* 2010)**. Hence the color change of baked cakes corresponds to the color of the raw material and oxidation reaction due pigments and polyphenols compounds present in pomelo fruit segments. These results clearly indicate that the addition of fruit segments has significantly decreased lightness and increased redness compared to control which is mainly influenced from the pomelo fruit segments.

Table 1 Quality characteristics of supplemented cake

Pomelo segments (%)	Weight (g)	Volume (cc)	Specific Volume (cc/g)	Density (g/cc)	Texture (g force)	Color Measurement L*	a*	b*	dE
0	379.70±0.88^c	880±0.90^e	2.31±0.89^d	0.43±0.99^b	1093±0.67^a	61.36±0.52^a	3.71±0.70^d	18.26±0.92^b	35.16±0.74^a
Fresh									
10	383.31±0.96^b	1090±0.77^c	2.84±0.86^b	0.35±0.65^d	973±0.56^b	58.24±0.86^a	4.37±0.55^c	18.53±0.65^b	37.24±0.49^a
20	385.20±0.64^b	1140±0.58^b	2.96±0.61^a	0.33±0.51^d	948±0.83^c	57.42±0.72^a	4.68±0.61^b	18.87±0.81^b	36.34±0.51^a
30	388.80±1.12^b	1189±0.75^a	3.05±0.94^a	0.32±0.74^d	875±0.95^e	56.68±0.97^a	5.11±0.73^a	19.11±0.59^a	37.06±0.63^a
Dry									
2.5	385.90±0.71^b	950±0.64^d	2.46±0.68^c	0.40±0.48^b	934±0.89^d	57.91±0.54^a	4.41±0.60^c	19.02±0.66^a	36.30±0.38^a
5	391.17±0.49^a	890±0.99^e	2.27±0.74^d	0.43±0.94^b	856±0.78^f	55.34±0.52^b	4.83±0.84^b	19.27±0.71^a	36.57±0.74^a
7.5	396.82±0.67^a	839±0.80^f	2.11±0.74^e	0.47±0.88^a	811±0.93^g	54.85±0.77^b	5.61±0.92^a	19.50±0.84^a	37.19±0.63^a

Note: Each column is shown as a mean±standard deviation (n=4);Values for different letters in the same column differ significantly (p0.05).

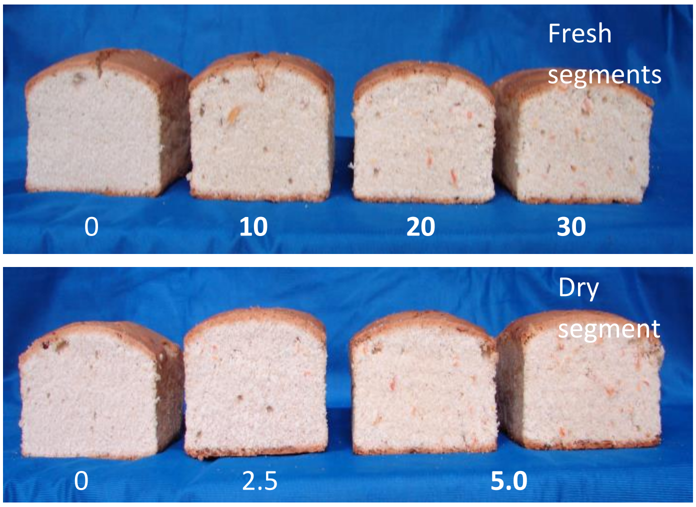


Figure 1 Cross section view of cakes supplemented with fresh and dry pomelo fruit Segments

Sensory evaluation of supplemented and unsupplemented cakes

Sensory evaluation score for the cakes fortified with different levels of pomelo fruit segments are illustrated in Table 2. The crust shapes of the samples and controls were not significantly different. On the other hand, the crust color and appearance varied greatly between the supplemented cakes ranging from 8.0-7.0. Increasing the level of supplemented fruit segments resulted in a slight drop in crumb color and texture (7.0-6.0), while there was not much significant difference in softness between the supplemented cakes. Taste slightly decreased in cakes supplemented with 30% of fresh segments. In case of dry segment supplementation, 5% and 7.5% received lower preference in comparison to the control. The supplementation of dry pomelo segments in higher amounts (5% and 7.5%) lead to the reduction of score due to the increased sourness and bitterness in cake compared to the other level of fortification. However, scores of cakes evaluated in terms of overall acceptability decreased significantly with increased level of substitution. The result clearly indicates that pomelo fruit segments in cake at levels 30% (fresh) and 5.0% (dry) has retained the fruit flavor and the bitterness was palatable. Our result was in accordance with **Qureshi *et al.* (2017)** wherein they have reported that incorporation of grapefruit albedo in cakes was found to be sensorially acceptable at 5.6% and in terms of sensory characteristics, panelists noted a slightly bitter aftertaste and darker color at highest level of fortification.

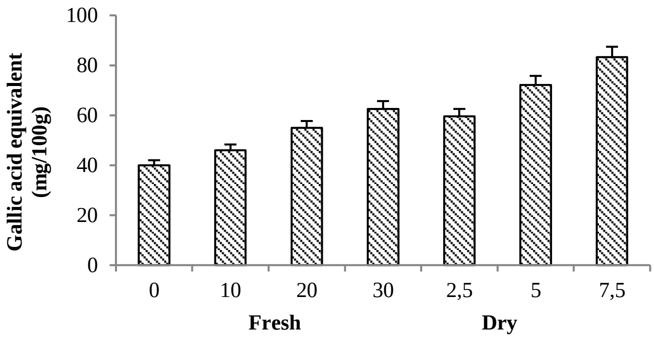
Table 2 Sensory analysis of fresh and dried fruit segments in cake

Pomelo segments (%)	Crust		Crumb			
	Color (9)	Shape (9)	Color (9)	Texture (9)	Taste (9)	Over all Acceptibility (9)
0	8.0±1.19[a]	8.0±0.76[a]	7.0±1.24[a]	7.0±1.70[a]	7.0±1.02[b]	7.0±1.76[a]
Fresh						
10	8.0±1.40[a]	8.0±0.88[a]	7.0±1.91[a]	7.0±1.83[a]	7.0±1.18[b]	7.0±1.56[a]
20	8.0±1.25[a]	8.0±1.17[a]	6.5±1.88[b]	7.0±1.41[a]	7.5±1.52[a]	7.0±1.72[a]
30	8.0±1.45[a]	8.0±0.93[a]	6.0±1.62[c]	7.0±1.19[a]	7.5±1.77[a]	6.5±1.51[ab]
Dry						
2.5	7.5±1.96[b]	8.0±1.24[a]	7.0±1.45[a]	7.0±1.56[a]	7.0±1.68[b]	7.0±1.96[a]
5	7.5±1.32[b]	8.0±1.39[a]	6.5±1.85[b]	6.5±1.29[b]	6.5±1.44[bc]	6.5±1.24[ab]
7.5	7.0±1.68[c]	8.0±0.99[a]	6.0±1.22[c]	6.0±1.33[c]	6.0±1.27[c]	5.5±1.67[c]

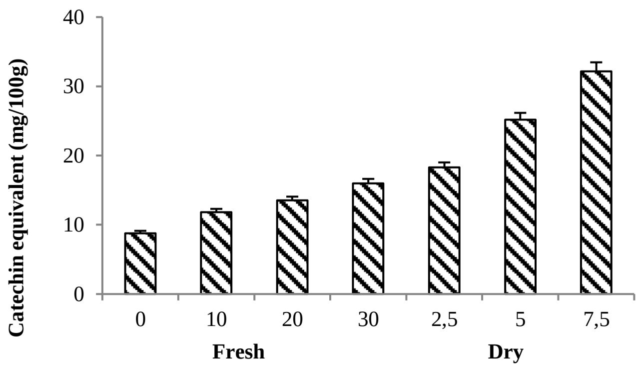
Note: Values in the parenthesis indicate maximum score; Values for a particular column followed by different letters differ significantly ($p < 0.05$); 9-point hedonic scale: 9-Like Extremely;8-Like Very Much; 7-Like Moderately; 6-Like Slightly; 5-Neither Like nor Dislike; 4-Dislike Slightly; 3-Dislike Moderately; 2-Dislike Very Much; 1-Dislike Extremely

Bioactive components in prepared cakes

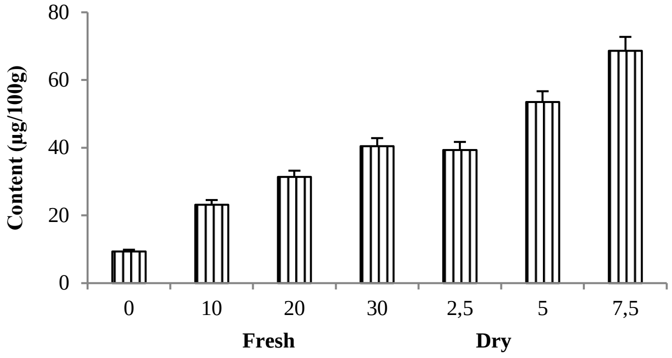
Bioactive compounds play a key role as drivers for health and functional foods. The supplemented cakes showed higher amounts of bioactive compounds compared to control cakes. The bioactives ranged from 49.19 to 81.52 mgGAE/100g for phenolics, 12.66 to 33.36 mg CE/100g for flavonoids and 24.33 to 67.24 µg/100g for carotenoids (Figure 2A, B and C). The addition of fruits segments in formulation has resulted in the increase in bioactive compounds depending upon the concentrations used during product preparation. Polyphenolic compounds (phenolics and flavonoids) and carotenoids function as antioxidants by chelating redox-active metal ions, inactivating radical chains, as well as preventing the creation of reactive oxygen radicals. It has been widely studied that total phenolic content correlates with antioxidant activity in a variety of foods **(Jayaprakasha & Patil, 2008)**. Thus, they are used in functional foods as natural antioxidant **(Viuda-Martos *et al.* 2011)**. There have also been reports of flavonoids and phenols exhibiting diverse biological activities such as antibacterial, anti-inflammatory, antioxidant, cytotoxic, anti-tumor, antispasmodic, and anti-depressant properties **(Ghasemzadeh & Ghasemzadeh, 2011)**. **Toh et al., (2013)** reported higher phenolics and flavonoid contents in juice of pomelo and further reported that the presences of these compounds are highly correlated with antioxidant properties. Thus, product rich in the spectrum of components are increasingly being used in the food industry for their synergistic effects and nutraceutical benefits. From this point of view, the cake supplemented with pomelo fruit segments may be considered as promising functional foods with value addition.



(A)



(B)



(C)

Figure 2 Bioactive compounds in cake supplemented with fresh/dry fruit segments (a) Phenolics (b) Flavonoids (c) Carotenoids

***Invitro* digestion method**

The cakes which was sensorialy acceptable *i.e.,* 30% fresh and 5% dry segment supplemented cakes were alone taken for this study to access its bioaccessibility efficiency. The percentage level showed a regular behavior with increased bioaccessibility in dry segment supplemented cake compared to fresh (Figure 3). After *invitro* digestion the phenolic content present in per serving was 79% for dry and 67% for fresh segment supplementation. Similarly, for flavonoid and carotenoids dry segment supplemented cake exhibited higher bioaccessibility with 64 and 50% compared to fresh segment supplementation with 40 and 32% respectively. From this results cake fortified with dry pomelo segments was found to have higher fruit content with enhanced in bioactive constituents, hence the bioaccessibility efficiency was found to be significantly increased reaching the greater value. **Zeng *et al.* (2016)** reported that higher the phenolic content in food sample higher will be the phenolic bioaccessibility which was accordance to our result. Phenolic are recognized as important bioactive because once released they get converted into low molecular weight metabolite and get absorbed into the blood and exert several health benefits such as against oxidative stress, cardiovascular disease, diabetes and cancer (**Amarowicz & Pegg, 2008; Villegas *et al.* 2008**). Hence, there is a growing recognition of the potential health benefits of foods rich in phenolics.

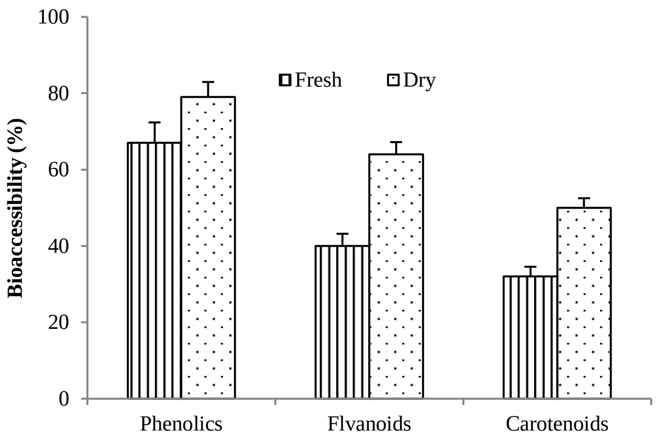


Figure 3 In vitro bioaccessibility of bioactives in cakes fortified with fresh (30%) and dried (5%) pomelo segments

Proximate composition of cake

The supplementation of pomelo fruit segments to wheat improved the chemical composition of the cakes as presented in Table 3. The cakes enriched with 30% fresh and 5% dry were sensorially preferred and analyzed further for their compositions. Cake substituted with pomelo fruit segments at different levels showed significant differences for total fat, protein and ash content relative to control cake. The moisture content of fresh and dry supplemented cake ranged from 14.74 and 12.72% compared to control (15.93%) that shows the certainty of prolonged shelf-life. Lin et al. 2017, pea protein fortified cakes showed a decrease in moisture content with high protein contents, which agreed with our findings. Increase starch and protein interaction resulted in the entrapment of water molecules through electrostatic forces, contributing to the decrease in moisture content with increasing substitution levels **(Zhang *et al.* 2016).** Thus, competition for water can generate a rigid structure by weakening the development of gluten-protein networks in wheat flour. The fat content decreased with the increasing concentrations of fruit segments from 35.0-32.0%. The protein and ash content increased from 6.50 and 0.78% (fresh) to 7.12 and 0.81% (dry) as against control (6.12 and 0.81%). The increase in protein content could be due to the substitution effect caused by pomelo fruit segments. **Abiodun *et al.* (2012)** reported that the protein content in samples contributes to the nutritional value of the human diet. Thus, substitution of pomelo fruit segments in cake decreased the moisture and fat content whereas it increased the protein and ash content compared to unsupplemented cake.

Table 3 Proximate composition (%) of supplemented cakes

Pomelo segments (%)	Moisture (%)	Fat (%)	Ash (%)	Protein (%)
0	15.93±1.13[a]	35.0±1.16[a]	0.80±0.77[a]	6.12±1.04[c]
Fresh (30%)	14.74±0.98[a]	34.0±1.22[a]	0.78±0.81[a]	6.50±1.11[b]
Dry (5%)	12.72±1.09[b]	32.0±1.02[a]	0.81±0.69[a]	7.12±1.25[a]

Note: Data is based on dry weight; Means are multiplied by standard deviations (n = 3); Significant differences for column values followed by different letters with p< 0.05

CONCLUSION

An innovative cake formulation featuring pomelo fruit segments has been developed. Physical characteristics indicated a reduction in cake volume for dry segment substitution and vis-versa for fresh segments. The color value showed increase in darkness and redness of the cake. By gradually increasing the level of fruit segments, the cake became richer in bioactive compounds. Increase in the amount of fruit segments in cakes decreased the sensory value. Cakes prepared at 30% and 5% were more acceptable and had no adverse effects on quality compared with that of control cakes. The bioaccessibility index was also found to be higher in cake supplemented with dry pomelo fruit segments showing greater value for phenolics followed by flavonoids and carotenoids. Increased substitution levels have also boosted the amount of protein and ash in the developed cake as compared against the control. These findings suggest that the cake developed with supplementation have better nutritional value with consumer acceptability

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