



Determination of Total Phenolics, Flavonoids, Carotenoids, β -Carotene and DPPH Free Radical Scavenging Activity of Biscuits Developed with Different Replacement Levels of Pumpkin (*Cucurbita maxima*) Peel, Flesh and Seeds Powders

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ABSTRACT

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Pumpkin (*Cucurbita maxima* Lam.) is a well-known, extensively grown and consumed crop, world-wide. Pumpkins are natural and rich source of potential bioactive compounds. The presence of active phytochemicals makes these fruits a great matrix to be further exploited for therapeutic purposes, beyond biotechnological applications. Peel, flesh and seeds of this fruit are heavily loaded with phenolics, flavonoids and carotenoids, which are the main tributes of this functional and medicinal food. Present study was designed to utilize these parts of pumpkin in the form of powders, at 0, 5, 10 and 15% replacement levels with white flour, to develop biscuits and to obtain methanolic extracts of these biscuits to determine their phytochemical parameters. Among the different treatment biscuits, highest amount of total phenolics (101.79 mg GAE/100 g), flavonoids (60.74 mg CE/ 100 g) and DPPH free radical scavenging activity (38.00 mg AAE/100 g) was found in biscuits with 15% replacement of pumpkin seeds powder, while biscuits with 15% replacement of pumpkin flesh powder exhibited highest amount of total carotenoid contents (6.95 mg/ 100 g) and β carotene (2.86 mg/100 g). Functional biscuits developed from replacement of pumpkin parts powders with wheat flour may be offered to patients facing oxidative stress, degenerative diseases and diabetes. These biscuits can be offered to children for better growth and development. Consumers awareness through proper marketing at commercial level with proper labelling of nutritional facts, may lead to increased demand of this functional and medicinal food rich in bio actives.

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Introduction

Pumpkins, members of family *Cucurbitaceae* and genus *Cucurbita*, have been used traditionally in treating diseases. These pharmacological activities of pumpkins are attributed to their excellent phytochemical composition. These *Cucurbita* species are natural source of phenols, carotenoids, tocopherols and terpenoids (Salehi et al., 2019). *Cucurbita* members are major economically important species of plants, with edible fruits and vegetables, possessing nutritional significance and when peel and pulp extracts of these fruits were tested with

different solvents and their different ratios, a significant amount of total phenolic contents, total flavonoid contents and antioxidant activity was found present in them (Sing et al., 2016). Pumpkin is well known fruit to possess high carotenoid and phenolic contents (Wahyono et al., 2019). Plants with high oxidant values could also be utilized as natural antioxidant sources (Kina et al., 2021). Bioactive compounds are very crucial for human nutrition and health as they prevent the development of many diseases (Algarni, 2020). Ethanolic extracts of plants like *Thymbra*

spicata, possess low levels of oxidant and high antioxidant potential (Mohammed et al., 2020). Seed-used pumpkin is a traditional, well-known crop mainly processed for seeds, about 50% portion of this crop is wasted directly in the fields, in the shape unused portion of peel and flesh. On the other hands pumpkin when cultivated for household consumption and industrial processing, seeds are usually wasted as bio waste material (Chen et al., 2019).

Pumpkin peel, flesh and seeds were evaluated for macronutrients, phenolic contents, flavonoids contents and antioxidant activity and results revealed that amounts of phytochemicals differ among parts of pumpkin (Abdo et al., 2020). According to Mala and Kurian (2016) peel and pulp portion of pumpkin are good source of nutrients as these parts exhibited a reasonable value of polyphenols and antioxidants, which suggested their usage in bakery and other food products as enrichment flours. Pumpkin seed flour was tested for its total phenolic contents and antioxidant activity and results proved that this flour could be used a nutritive ingredient in muffins offered to children (Bialek et al., 2016). Pumpkin peel and pulp were investigated for phytochemical compounds and appreciable amounts of β carotene, total phenols and total phenolics were present (Saleh et al., 2019). Pumpkin seeds and rind are well balanced with phenolic compounds, fiber and antioxidant activity (Nyam et al., 2013). Various solvents used for extraction of pumpkin skin and pulp were acetone, ethanol, methanol and water, and these extracts exhibited a promising range of total phenolic contents, flavonoid contents and antioxidant activity (Babiker et al., 2018). Hussain et al. (2021) performed phytochemicals analysis of pumpkin peel, flesh and seeds powder's methanolic extracts, the total phenolic contents in peel, flesh and seeds were found 93.40, 134.59 and 224.61 mg GAE/100 g powder, the total flavonoid contents in these three parts were found 45.0, 77.11 and 139.37 mg CE/100 g powder, the total carotenoids in peel, flesh and seeds were found 23.7, 35.2 and 8.2 mg/100 g powder and quantity of β carotene in these parts was found 4.60, 6.18 and 0.99 mg/100 g powder, respectively, which, clearly shows that each part of pumpkin is possessed with sufficient amount of these bio actives.

Drying of pumpkin at higher temperature retained maximum phenolic compounds and antioxidant activities of pumpkin flour (Bochnak and Swieca 2020). Kvapil et al. (2021) osmotically dehydrated pumpkin and studied the effect of process conditions on quality indices of pumpkin. Jukic et al. (2018) evaluated the quality of biscuits developed from different blends of wheat flour and flour obtained from press cake of seeds after oil extraction. Biscuits prepared with blends of pumpkin seed flour and oats powder were high in nutritional aspects, particularly carotenoids contents and antioxidant activity of blended biscuits was up to the mark. As biscuits are food of mass consumption, consumed by every group of age and easy to prepare so, these baked items can be used as a tool to incorporate nutritionally rich powders and flours of fruits and vegetables, especially pumpkin to develop functional foods with increased phytonutrients (Das et al., 2021). Bakery products such as biscuits and cookies are widely consumed all around the world but these items do not have an up to the mark nutritional status and enrichment of these food products with minerals, vitamins, phenolics, carotenoids, natural colorants and fibers may be proceeded

with powdered fruits and vegetables (Salehi, 2019). Keeping in view the mentioned facts about phytochemistry of pumpkin parts and the possible uses of pumpkin in food products, the present study was designed to develop nutritional biscuits using different replacement levels of pumpkin peel, flesh and seeds, and to determine phytochemicals present in these biscuits.

Material and Methods

Preparation of Pumpkin Powders, Biscuits and Extracts

Mature pumpkin fruits (n = 50) and raw materials for biscuit preparation were purchased from local market of Sargodha, Pakistan. Reagent grade chemicals used in this study were purchased from Sigma-Aldrich chemicals. Pumpkins were cut with knife and the three fractions in the form of peel, flesh and seeds were divided, cleaned and dried in oven with hot air flow (Hitachi HOTG-52 Japan) at 60°C until constant weight was achieved, then grinded to obtain fine powders, as described by Hussain et al. (2021). Biscuits with 100% white flour (control) and with 5, 10 and 15% replacement levels of pumpkin peel, flesh and seeds powders were prepared by adopting the method elaborated by Kulkarni and Joshi (2013) with some modifications. Extracts of prepared biscuits were obtained using 80% methanol as solvent by adopting the procedure given by Asif et al. (2017) with some modifications. Briefly, 20 g of biscuits from each treatment were soaked in 200 mL of methanol (80%) and solution was continuously stirred at 200 rpm for a time period of 120 hrs at orbital shaker, at room temperature and the next step was separation of mixture through filtration technique using Whatman filter paper. These filtrates were concentrated using vacuum rotary evaporator at 45°C. Exact five milliliters of every extract were stored at 4°C to perform further analysis.

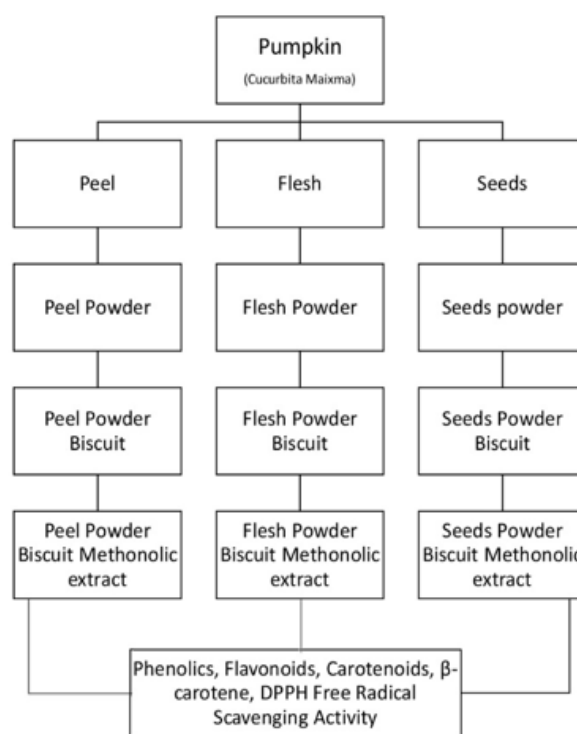


Figure 1. Graphical diagram of overall research work plan

Table 1. Total Phenolics (mg GAE/100 g) of biscuits developed with different replacement levels of pumpkin parts (peel, flesh, seeds) powder's biscuits

Level of replacement	Total Phenolics (mg GAE/100 g)				Over all Means
	White flour	Peel powder	Flesh powder	Seeds powder	
0% (Control)	65.63±0.18h	-	-	-	-
5%	-	68.01±0.12g	70.13±0.55f	84.41±1.02c	74.19±2.60C
10%	-	73.23±0.56e	76.33±0.95d	92.49±0.69b	80.68±3.01B
15%	-	78.03±0.55d	82.49±0.61c	101.79±0.99a	87.43±3.66A
Over all Means	-	73.09±1.46C	76.32±1.82B	92.90±2.55A	

Numerical values with same alphabetical letters in a column are statistically non-significant while, numerical values with different alphabetical letters in a column are statistically significant. Small alphabetical letters present comparison among individual mean values while, capital alphabetical letters represent comparison among overall mean values.

Table 2. Total Flavonoids (mg CE/100 g) of biscuits developed with different replacement levels of pumpkin parts (peel, flesh, seeds) powder's biscuits

Level of replacement	Total Flavonoids contents (mg CE/100 g)				Over all Means
	White flour	Peel powder	Flesh powder	Seeds powder	
0% (Control)	32.44±0.13j	-	-	-	-
5%	-	35.99±0.09i	37.72±0.17h	43.39±0.10d	39.03±1.12C
10%	-	39.74±0.22g	42.93±0.05e	51.21±0.14b	44.63±1.71B
15%	-	42.38±0.16f	47.17±0.08c	60.74±0.10a	50.10±2.75A
Over all Means	-	39.37±0.93C	42.61±1.37B	51.78±2.51A	

Numerical values with same alphabetical letters in a column are statistically non-significant while, numerical values with different alphabetical letters in a column are statistically significant. Small alphabetical letters present comparison among individual mean values while, capital alphabetical letters represent comparison among overall mean values.

Table 3. DPPH free radical scavenging activity (mg AAE/100 g) of biscuits developed with different replacement levels of pumpkin parts (peel, flesh, seeds) powder's biscuits

Level of replacement	DPPH free radical scavenging activity (mg AAE/100 g)				Over all Means
	White flour	Peel powder	Flesh powder	Seeds powder	
0% (Control)	6.91±0.05i	-	-	-	-
5%	-	17.80±0.09f	14.38±0.08h	26.74±0.22c	19.64±1.84C
10%	-	20.69±0.05e	16.61±0.17g	32.16±0.13b	23.15±2.33B
15%	-	24.18±0.11d	18.04±0.13f	38.00±0.51a	26.74±2.96A
Over all Means	-	20.89±0.92B	16.34±0.54C	32.30±1.63A	

Numerical values with same alphabetical letters in a column are statistically non-significant while, numerical values with different alphabetical letters in a column are statistically significant. Small alphabetical letters present comparison among individual mean values while, capital alphabetical letters represent comparison among overall mean values.

Table 4. Total Carotenoids (mg/100 g) of biscuits developed with different replacement levels of pumpkin parts (peel, flesh, seeds) powder's biscuits

Level of replacement	Total Carotenoids (mg/100 g)				Over all Means
	White flour	Peel powder	Flesh powder	Seeds powder	
0% (Control)	2.17±0.018j	-	-	-	-
5%	-	3.34±0.019g	4.42±0.044e	2.64±0.041i	3.46±0.260C
10%	-	4.81±0.021d	5.82±0.067b	2.98±0.043h	4.54±0.417B
15%	-	5.47±0.057c	6.95±0.050a	3.72±0.043f	5.38±0.467A
Over all Means	-	4.54±0.315B	5.73±0.367A	3.11±0.162C	

Numerical values with same alphabetical letters in a column are statistically non-significant while, numerical values with different alphabetical letters in a column are statistically significant. Small alphabetical letters present comparison among individual mean values while, capital alphabetical letters represent comparison among overall mean values.

Table 5. β carotene (mg/100 g) of biscuits developed with different replacement levels of pumpkin parts (peel, flesh, seeds) powder's biscuits

Level of replacement	β carotene (mg/100 g)				Over all Means
	White flour	Peel powder	Flesh powder	Seeds powder	
0% (Control)	0.10±0.006i	-	-	-	-
5%	-	0.56±0.015e	1.14±0.026d	0.20±0.007h	0.64±0.137C
10%	-	1.13±0.015d	2.00±0.029b	0.33±0.009g	1.15±0.241B
15%	-	1.78±0.017c	2.86±0.019a	0.50±0.012f	1.71±0.341A
Over all Means	-	1.16±0.176B	2.00±0.248A	0.34±0.043C	

Numerical values with same alphabetical letters in a column are statistically non-significant while, numerical values with different alphabetical letters in a column are statistically significant. Small alphabetical letters present comparison among individual mean values while, capital alphabetical letters represent comparison among overall mean values.

Determination of Total Phenolic Contents

The total phenolic contents in control biscuits and biscuits prepared with different replacement level of pumpkin parts powders were determined according to the procedure of Shen et al., (2009) with some modifications. Accurately 0.5 mL of each extract, 2.5 mL of Folin-Ciocalteu reagent (100 g/kg) and 2 mL of NaCO₃ (75 g/kg) were added into a tube and mixed well. This mixture was given a stay time of 15 minutes at 50°C. Then the absorbance was measured at 760 nm using spectrophotometer (Hitachi U-2001, Hitachi Instruments Inc. Tokyo, Japan), against methanol water solution (80:20, v: v) as blank solution. The results for total phenolic contents of sample biscuits were expressed as mg/100 g biscuits of gallic acid equivalent (GAE).

Determination of Total Flavonoid Contents

The total flavonoid contents in biscuits were determined by following the method adopted by Shen et al., (2009) with some modifications. Briefly describing, extract of each sample, exactly 1 mL and 0.3 mL NaNO₃ (50 g/kg) were mixed homogeneously in a test tube, followed by the addition of 0.3 mL Al (NO₃)₃ (100 g/kg) and 4 mL NaOH (50 g/kg). Stay time of 15 minutes was given to the solution and then absorbance was measured at 510 nm using spectrophotometer, against methanol water solution (80:20, v: v) as blank solution. The total flavonoid contents of the samples were expressed as mg/100 g biscuits of Catechin equivalent (CE).

Determination of DPPH Free Radical Scavenging Activity

Free radical scavenging activity of biscuits was analyzed through DPPH assay as illustrated by Brand-Williams et al. (1995), with slight modifications. An amount of 0.01 g of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was poured to a 25 mL flask having solvent (80:20 methanol/water (v/v)). A calibration curve of ascorbic acid was also established. An amount of 100 µL from each type extract was collected in microplates, then an amount of 2.0 mL solvent and 250 µL DPPH reagent were added. These microplates were shaken and kept in darkness at ambient temperature for a time period of 20 mint. Reduction in DPPH absorbance by spectrophotometer, at 520 nm was witnessed every 5 min intervals unless this absorbance was stabilized after a time period of 30 mints. Methanol played its role as blank solution, while DPPH solution with no test samples acted as control. The results were expressed as mg of ascorbic acid equivalent (AAE) /100 g powder in a reaction time of 30 mints.

Determination of Total Carotenoid Contents

Determination of total carotenoids in biscuits was carried by following the method adopted by De Carvalho et al., (2012) with slight changes. Control biscuits and biscuits developed with different ratios of pumpkin parts 25 g each, was taken in vessel covered with aluminum foil. Exact 80 mL of extraction solvent, acetone/n-hexane (1:1, v/v) was added to the vessel and shaken well. Through separation technique release of organic phase was carried out. Again 15 mL of acetone/n-hexane (1:1, v/v) was added to repeatedly carry out the aqueous phase extraction until the colorless appearance was achieved. Anhydrous sodium

sulphate was used to dehydrate the organic phase followed by the spectrophotometric analysis of extracts at 450 nm. Total carotenoids were expressed as mg/100 g of biscuits.

Determination of β Carotene

β carotene in biscuit samples was determined by following the method adopted by De Carvalho et al., (2012) with slight modifications. HPLC with UV/visible photodiode was used to determine β contents. Reverse phase carbon-30 column having length 250 mm with an internal diameter of 4.6 mm was used. Mobile phase used in this experiment was 80% methanol and 20% tertiary butyl methyl ether, with flow rate of 0.8 mL/min, while the sample size injected was 25 µL. Analysis was completed in a run time of 60 minutes at 30°C. β carotene contents were expressed as mg/ 100 g biscuits.

Results and Discussion

Total Phenolic Contents Analysis of Added Pumpkin Peel, Flesh and Seeds Powders Biscuits

Mean values for total phenolic contents analysis of 100% white flour biscuits and biscuits prepared with different replacement levels of pumpkin parts (peel, flesh and seeds) have been accessible in Table 1. From these results it was evident that higher values of total phenolics were observed in biscuits having 15% seeds powder and lowest amount total phenolics was present in control biscuits. In biscuits developed by using pumpkin peel and flesh powders the total phenolic contents were relatively lower than in biscuits developed using pumpkin seeds powders. From these results it was also obvious that total phenolic contents in biscuits were found increasing significantly with increasing level of all three types of pumpkin powders.

Que et al. (2008) reported that during drying of pumpkin, high temperature leads the formation of phenolic compounds. Aydin and Gocmen (2015) reported that development of pumpkin flour by drying has a positive impact on total phenolic compounds. Zdunic et al. (2016) experimentally checked total phenolics in fresh pumpkin fruit (*Cucurbita maxima*) and traditional pumpkin products and expressed the results on fresh mass basis. Total phenolics in pumpkin fruit were, 905.9 µg GAE/g, in pumpkin sweet in wine 227.2 µg GAE/g pumpkin jam 769.1 µg GAE/g and in pumpkin juice 93.0 µg GAE/g. As expected, there were lower but still significant amounts of total phenolic contents in pumpkin products as compared to fresh pumpkin and this was probably due to thermal processing. Das et al. (2021) prepared biscuits with different proportions of pumpkin seeds powder and oats powder and concluded that increased percentage of pumpkin seeds powder resulted in increased total phenolic contents of biscuits. Rozylo et al. (2014) made wheat bread by adding different levels of fresh pumpkin (*Cucurbita maxima*) pulp directly into wheat flour and determined total phenolic contents of bread buffer extracts and extracts after digestion. In control bread buffer extracts total phenolic contents were found 6.3 mg/g and replacement of 5% pumpkin pulp, no significant difference was observed in total phenolic contents as value was obtained 6.1 mg/g but 10% replacement of pumpkin pulp caused a significant increase in total phenolic contents of bread as value was

obtained 7.3 mg/g and again increase in replacement level of pumpkin pulp from 15% to 20% resulted non-significant data of total phenolic contents of bread as values were 6.8 mg/g and 7.2 mg/g respectively. Wahyono et al. (2019) prepared bread with different replacement levels of pumpkin flour against wheat flour and observed a significant increase in total phenolics of bread with increased level of pumpkin flour. The amount of total phenolics in bread with 100% wheat flour was found 1.38 mg GAE/g, which were raised to 5.39 mg GAE/g in bread developed with 20% pumpkin flour.

Mojarad and Mahdikhani (2020) studied the effect of replacement of pumpkin powder at different levels, on the preparation of biscuits and observed a significant increase in total phenolics of biscuits with increased levels of pumpkin flour. Algarni, (2020) developed sponge cake and pan cake fortified with pumpkin flour and determined total phenolic contents in these baked products prepared with different replacement levels of pumpkin flour. As pumpkin flour level was increased from 5 to 20%, total phenolic contents were increased from 7.50 to 16.18 mg GAE/g, respectively in these blends. Pasanda et al. (2019) formulated composite flours by adding pumpkin flour in wheat flour to develop biscuits and observed a significant increase in total phenolics of formulated biscuits as compared to wheat flour biscuits. Malkanthi and Hiremath (2020) successfully supplemented pumpkin pulp powder into the traditional string hoppers and noticed a significant increase in total phenolics. Zaki et al. (2018) incorporated pumpkin powder in wheat flour to develop cake and a significant increase in total phenolics of cake was observed with 15% level of pumpkin powder. Another member of *Cucurbitaceae* family, melon, peel and seeds flour incorporation in wheat flour cookies resulted significant increase in total phenolic contents of cookies (Ertas and Aslan 2020).

From the past studies different examples are present that addition of fruits peel in biscuits provided better total phenolic contents as compared to simple wheat biscuits. Ismail et al. (2014) added pomegranate peel powder in straight grade flour in different ratios and determined total phenolic contents of cookies. In cookies with 100% straight grade flour total phenolics were found 90.70 mg GAE/100 g and a significant increase in total phenolic contents of cookies was observed as replacement level of pomegranate peel flour was increased. Ajila et al. (2008) incorporated mango peel powder in soft dough biscuits and studied effect on phenolic contents of biscuits. In control biscuits total polyphenolic contents were present 540 µg GAE/g and addition of mango peel powder up to 20% increased these polyphenolic contents to a significant value of 4500 µg GAE/g so these results justified the statement that fruits peels are excellent sources of polyphenolics. Nyam et al. (2013) used pumpkin rind and seeds powder as bakery product ingredient to develop bread and came on to conclusion that 5% replacement of pumpkin rind and seeds powder raised the total phenolic of bread as compared to control bread.

Total Flavonoids Contents Analysis of Added Pumpkin Peel, Flesh and Seeds Powders Biscuits

Numbers of total flavonoid contents analysis of 100% white flour biscuits and biscuits prepared with different replacement levels of pumpkin peel, flesh and seeds

powders, has been given in Table 2. Control biscuits developed with 100% white flour presented lowest total flavonoid contents whereas highest total flavonoid contents were observed in biscuits developed with 15% pumpkin seeds powder. From these results it was evident that total flavonoid contents were found increasing significantly with increasing level of all three types of pumpkin powders.

Valenzuela et al. (2014) reported that flavonoids are the most abundant and available phenolic contents in pumpkin. Singh et al. (2016) used different solvents in different ratios to determine total flavonoid contents in pumpkin two parts (peel and pulp) and observed significant amounts of these phytochemicals. Rozylo et al. (2014) made wheat bread by adding different levels of fresh pumpkin (*Cucurbita maxima*) pulp directly into wheat flour and determined total flavonoid contents of bread buffer extracts and extracts after digestion. In control bread buffer extracts total flavonoid contents were found 2.8 mg/g and replacement of 5% pumpkin pulp no significant difference was observed in total flavonoid contents as value was obtained 2.6 mg/g but 10% replacement of pumpkin pulp caused a significant increase in total flavonoid contents of bread as value was obtained 3.9 mg/g but an increase in replacement level of pumpkin pulp from 15% to 20% resulted a significant decrease of total flavonoid contents of bread as values were 1.91 mg/g and 1.96 mg/g respectively. Alshehry, (2020) provided study upon the preparation and nutritional properties of cookies with partial replacement of pumpkin seeds powder and reported that pumpkin seed had great amount of total phenolic and flavonoid contents.

From past studies there are several examples present that addition of pumpkin in different food products enhance the total flavonoid contents. Odunlade et al. (2017) evaluated the effect of fortification of maize porridge with pumpkin leaves extracts and observed a significant increase in total flavonoid contents. Abou El Samh et al. (2013) improved the flavor and nutritional value of yogurt by adding pumpkin at different concentrations and observed a significant increase in total flavonoid contents. Barakat and Hassan (2017) prepared stirred yogurt with three different varieties of pumpkin pulp and noticed a significant increase in total flavonoid contents of this dairy product.

DPPH Free Radical Scavenging Activity Analysis of Added Pumpkin Peel, Flesh and Seeds Powders Biscuits

DPPH free radical activity analysis of control biscuits and biscuits prepared with different replacement levels of pumpkin fractions powders has been fetched in Table 3. From these results it was evident that replacement of seeds powder exhibited higher DPPH free radical scavenging activity as compared to peel and flesh powders and highest antioxidant activity was observed in biscuits having 15% seeds powder. It was also observed that and this DPPH free radical scavenging activity was found increasing significantly with increasing level of all three types of pumpkin powders.

Total phenolic contents are directly proportional to DPPH free radical scavenging activity, as pumpkin parts are rich source of total phenolic contents, they when added in biscuits enhanced the DPPH free radical scavenging

activity of biscuits. Kaur and Sharma (2017) prepared nutritional cookies by replacing 0, 15, 30 and 45% pumpkin seeds flour (both raw and roasted) with wheat flour and declared that 30% replacement level is most accepted for development of cookies. They observed significantly different antioxidant activity of all treatment cookies. Antioxidant activity of accepted cookies prepared with 30% replacement level of raw pumpkin seeds flour was $58.10 \pm 0.006\%$ and in control cookies with 100% white flour antioxidant activity was lower $49.50 \pm 0.006\%$. Mojarad and Mahdikhani (2020) studied the effect of replacement of pumpkin powder at different levels, on the preparation of biscuits and observed a significant increase in antioxidant activity of biscuits with increased levels of pumpkin flour. Algarni, (2020) developed sponge cake and pan cake fortified with pumpkin flour and determined the antioxidant activity of these baked products prepared with different replacement levels of pumpkin flour. As pumpkin flour level was increased from 5 to 20%, DPPH free radical scavenging activity was increased from 69.24 to 81.59%, respectively in these blends.

Pasanda et al. (2019) formulated composite flours by adding pumpkin flour in wheat flour to develop biscuits and observed a significant increase in antioxidant activity biscuits developed with composite flour containing pumpkin flour, as compared to simple wheat flour biscuits. Zaki et al. (2018) incorporated pumpkin powder in wheat flour to develop cake and a significant increase in DPPH free radical scavenging activity of cake was observed with 15% level of pumpkin powder. Indrianti et al. (2021) stated that pumpkin flour effects positively on antioxidant activity of flat tuber noodles as antioxidant activity of noodles significantly increased with increasing level of pumpkin powder. Malkanthi and Hiremath (2020) successfully supplemented pumpkin pulp powder into the traditional string hoppers and noticed a significant increase in antioxidant activity. DPPH free radical scavenging activity of a medicinal plant, *Euphorbia eriophora* was determined and it was concluded that this natural plant can be used as a natural antioxidant agent in different products (Akgul et al., 2022).

Das et al. (2021) prepared biscuits with blends of pumpkin seeds powder and oats powder and observed increased DPPH free radical scavenging activity in biscuits with increased proportion of pumpkin seeds powder. Wahyono et al. (2019) expressed after the experiments that flour from pumpkin when added to the bread significantly increased the antioxidant activity bread. The maximum antioxidant activity (76.59%) was exhibited by bread developed with 20% replacement of pumpkin powder with wheat flour, while the control bread prepared with 100% wheat flour exhibited relatively lower (62.10%) antioxidant activity. Nyam et al. (2013) used pumpkin rind and seeds powder as bakery product ingredient to develop bread and came on to conclusion that 5% replacement of pumpkin rind and seeds powder raised the DPPH free radical scavenging activity of bread as compared to control bread.

Another member of *Cucurbitaceae* family, melon, peel and seeds flour incorporation in wheat flour cookies resulted significant increase in antioxidant activity of cookies (Ertas and Aslan 2020). Several examples are there that addition of plant materials to baked goods gave rise to

antioxidant activity of these food products. Choi, (2009) prepared pine needle cookies and stated that DPPH free radical scavenging activity of cookies with 100% wheat flour was low (6%) and addition of up to 5% pine needle flour resulted significant increase in DPPH free radical scavenging activity of cookies (60%). He also concluded that more total phenolic contents result in more antioxidant activities in cookies. Ismail et al. (2014) added pomegranate peel powder in straight grade flour in different ratios and determined DPPH free radical scavenging activity of cookies. In cookies with 100% straight grade flour DPPH free radical scavenging activity was found 27.30% and a significant increase in DPPH free radical scavenging activity of cookies was observed as replacement level of pomegranate peel flour was increased. Uysal et al. (2021) determined that plants could be used as natural antioxidant sources.

Total Carotenoids Analysis of Added Pumpkin Peel, Flesh and Seeds Powders Biscuits

Analysis of total carotenoid contents of control biscuits and biscuits with different replacement levels of pumpkin parts powders was performed and data collected has been presented in Table 4. The findings revealed that highest total carotenoid contents were observed in biscuits having 15% flesh powder and lowest were found in control biscuits. Pumpkin peel and seeds incorporated biscuits presented relatively lesser total carotenoid contents as compared to flesh incorporated biscuits. It was also clear from the findings that these total carotenoid contents were found increasing significantly with increasing level of replacement of pumpkin powders.

Supportive results were obtained when Kaur and Sharma (2017) prepared nutritional cookies by replacing 0, 15, 30 and 45% pumpkin seeds flour (both raw and roasted) with wheat flour and declared that 30% replacement level is most accepted for development of cookies. They observed significantly different carotenoids contents in all treatment cookies. Total carotenoids contents of accepted cookies prepared with 30% replacement level of raw pumpkin seeds flour were 0.231 ± 0.0006 mg/100 g and in control cookies total carotenoids contents were lower with a value of 0.019 ± 0.0006 mg/100 g. Pasanda et al. (2019) formulated composite flours by adding pumpkin flour in wheat flour to develop biscuits and observed a significant increase in total carotenoid contents of pumpkin flour incorporated biscuits as compared to simple wheat flour biscuits.

Results similar to our study were obtained when Gurung et al. (2016) performed analysis to check quantity of total carotene (mg/100 g) of biscuits in which wheat flour was replaced by pumpkin puree at different levels. In control cookies total carotene was not detected but 10% replacement of wheat flour with pumpkin puree gave value of total carotene 2.38 mg/100 g and when this replacement level was increased to 20, 30 and 40% a significant increase in total carotene contents of biscuits was observed as values were 5.13, 11.01 and 14.55 mg/100 g. Das et al. (2021) prepared biscuits with blends of pumpkin seeds powder and oats powder and noticed higher total carotenoid contents in biscuits with greater percentage of pumpkin seeds powder.

Zdunic et al. (2016) performed some analyses to determine the total carotenoids in fresh pumpkin fruit (*Cucurbita maxima*) and traditional pumpkin products and expressed the results on fresh mass basis. Total carotenoids contents in pumpkin fruit were 86.3 µg/g, in pumpkin sweet in wine 27.6 µg/g pumpkin jam 63.9 µg/g and in pumpkin juice 28.6 µg/g. As expected, there were lower but still significant amounts of total carotenoids contents in pumpkin products as compared to fresh pumpkin and this was probably due to thermal processing. Provesi and Amante (2015) stated that loss or decrease in total carotenoids contents of pumpkin during processing may be due to isomerization or oxidation which effect biological activity and color. Temperature and oxygen exposure are important factors of carotenoids loss.

Ajila et al. (2008) incorporated mango peel powder in soft dough biscuits and studied effect on total carotenoid contents of biscuits. In control biscuits total carotenoid contents were present 17 µg/g and addition of mango peel powder up to 20% increased these carotenoid contents to a significant value of 247 µg/g and these results witnessed that fruit peels are healthy sources of carotenoids.

Analysis of β Carotene in Added Pumpkin Peel, Flesh and Seeds Powders Biscuits

Mean values for β -carotene analysis of 100% white flour biscuits and biscuits prepared with different replacement levels of pumpkin three fraction's powders have been given in Table 5. The findings revealed that among all the sample biscuits, highest β carotene content was observed in biscuits having 15% flesh powder and lowest β carotene value was found in control biscuits. Biscuits developed with pumpkin seeds powders were equipped with lesser β carotene contents, when compared with biscuits developed with flesh and peel powder. However, this β -carotene level was found increasing significantly with increasing level of replacement of each pumpkin fraction powder.

Results similar to our study were obtained when Pongjanta et al. (2006) determined different carotenoid contents including β carotene in pumpkin added bakery products and observed a significant increase in β carotene by increasing the replacement level of pumpkin powder as in control biscuits value of β carotene was found 0.06 mg/100 g and when 10% pumpkin powder was replaced with wheat flour this contents were increased to 0.15 mg/100 g, a further increase of replacement level up to 20% resulted an increase to 0.20 mg/100 g and when 50% pumpkin powder was replaced with wheat flour to develop biscuits β carotene was found 0.54 mg/100 g. Malkanthi and Hiremath 2020 successfully supplemented pumpkin pulp powder into the traditional string hoppers and noticed a significant increase in β carotene. Zaki et al. (2018) incorporated pumpkin powder in wheat flour to develop cake and a significant increase in β carotene of cake was observed with 15% level of pumpkin powder.

Supportive results were obtained when Dhiman et al. (2018) prepared cookies with different replacement levels of pumpkin powder and pumpkin seed kernel powder. Addition of pumpkin powder caused a significant increase in β carotene contents of treatment cookies as values of β carotene (mg/100 g) in control cookies, 10, 20 and 30% pumpkin powder cookies were 3.22, 4.13, 4.39 and 4.88

respectively. Similarly, addition of pumpkin seeds powder caused a significant increase in β carotene contents of treatment cookies as values of β carotene (mg/100 g) in control cookies, 10, 20 and 30% pumpkin powder cookies were 3.22, 3.58, 3.66 and 3.78 respectively. Malkanthi et al. (2018) prepared pumpkin seeds powder blended biscuits and determined β carotene of biscuits having 5% powder of pumpkin seeds as 0.51 mg/100 g. Toan and Thuy (2018) produced high quality pumpkin flour and produced biscuits from this flour. They determined β carotene in biscuits prepared from pumpkin flour as 4.02 mg/100 g. Das et al. (2021) witnessed higher values of β carotene in biscuits developed with greater percentage of pumpkin seeds powder.

Conclusion

Pumpkin is an important vegetable consumed across the world, which has great potential to be used as functional and medicinal food. Every fraction of pumpkin is loaded heavily with phytochemicals. Biscuits developed with different replacement levels of pumpkin peel, flesh and seeds were when analyzed for phytochemicals it was concluded that total phenolics, flavonoids, carotenoids, β carotene and DPPH free radical scavenging activity was found increasing significantly with increasing the level of replacement of pumpkin parts. Among the different treatment biscuits highest amount of total phenolics (101.79 mg GAE/100 g), flavonoids (60.74 mg CE/ 100 g) and DPPH free radical scavenging activity (38.00 mg AAE/100 g) was found in biscuits with 15% replacement of pumpkin seeds powder, while biscuits with 15% replacement of pumpkin flesh powder exhibited highest amount of total carotenoid contents (6.95 mg/ 100 g) and β carotene (2.86 mg/100 g).

Recommendations

Dominance of phytochemicals in all three fractions of pumpkin attracts the researchers and scientists to utilize this economical crop in development of functional food products. Beneficial ingredients from pumpkin can be extracted, isolated and utilized in development of medicines and drugs for the therapeutic purposes. Need of the time is to develop innovative food products using pumpkin peel, flesh and seeds, for the wellness of mankind and more important is the commercialization of these functional food products. Enrichment of pumpkin in meat products, beverages and confectionary industry will not only rise the production of this under-utilized crop but also its utilization at commercial level.

Conflict of Interest

The authors have declared no conflicts of interest for this article.

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