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ORIGINAL RESEARCH PAPER

EFFECT OF ANTIOXIDANTS AND DIETARY FIBER FROM APPLE AND STRAWBERRIES ON VALUE ADDITION INTO MUTTON PATTIES

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Superfruits like apples and strawberries are rich in dietary fibres and antioxidants and prevent the risk of many diseases. In this study, antioxidant content, total phenolic content and ferric reducing power analyses were carried out for the pulped and freeze dried fruit powders. From the tests, it was observed that apple peel had a high total phenolic content. The antioxidant content was high in apple peel and strawberries. The mutton patties were incorporated with the powders of apple peel, apple pulp and strawberry fruit. The Total dietary fibre, peroxide value tests, proximate analysis, colour analysis, microbiological studies, sensory analysis and storage studies were carried out and noticeable results were obtained as compared to the control and proved the increasing quality of the mutton patties.

Keywords: mutton patties, apple peel, apple pulp, strawberry, antioxidant, dietary fibre

Introduction

Fruits like apples and strawberries are found to have high antioxidant, fibre content. Apples are found to have high cellulose, while strawberries are the most rich in lignin (Rosset *et al*., 1985). Meat products are gaining high value on the market for their delicious taste. But the disadvantages are more due to their high cholesterol and low fibre content (Boyer and Liu, 2004). The fatty acids in meat undergo oxidation, reducing the meat flavour, taste and nutritional value. The recent trend of functional foods is gaining good response on the market. Functional foods increase the quality and nutritional content of the food and thereby reduce the risk of various diseases. Different results from the past have revealed that food rich in antioxidants and fibre will help in reducing the risk of various cardiovascular and cancerous diseases along with diabetes and obesity (Verma *et al*., 2013).

Meat is highly prone to oxidation due to its high lipid and protein content. This oxidation can have a deleterious change on human health as well on the quality, flavour, nutrition, texture and taste of meat (Valesco and William, 2011). Peroxides

are primary products of lipid oxidation, which are further decomposed into secondary products that affect the meat quality and the health of human beings (Fiori *et al*, 2013). The oxidation process can cause a detrimental effect not only on the lipid content but also on the protein content of meat. Therefore, the delay of oxidation is crucial to prolong the shelf life of meat. Oxidation leads to the production of free radicals which react with the muscles of the meat destroying protein and other nutrient content.

To supress oxidation and to add nutritional value to the meat, antioxidants and dietary fibre have to be added. Synthetic antioxidants such as BHA and BHT are being used in meat products but recently consumers are more health conscious of the use of artificial additives (Das *et al*., 2013). Hence, the necessity of using natural antioxidants has come into the light to improve the quality and nutritional value of meat and extend its shelf life. Plant-based phytochemicals, such as antioxidants and flavonoids, from fruits, have good radical scavenging property which lengthens the shelf life by reducing the process of oxidation in meat (Boyer and Liu, 2013).

The phytochemicals present in apples such as carotenoids, vitamins, flavonoids, quercetin, catechin, phloridzin and chlorogenic acid act as strong antioxidants. Similarly, strawberries are considered to be “super foods” because of their antioxidant content. Not just do they increase the antioxidant effect in meat but also enrich its colour. Strawberries are found to contain high antioxidants, vitamin C and polyphenols such as flavonols, anthocyaninsand other essential phenolic acids such as ellagitannins and ellagic acid (Cerda *et al*., 2005). Incorporation of fruits with high polyphenols in meat products would enhance their physiological, functional and nutritional value (Buricova *et al*., 2011).

The objective of this study is to investigate the effectiveness of the value addition of functional mutton supplemented with apple and strawberry powder and its effect on the various physicochemical, microbiological, colour, textural and sensory characteristics of the product. With the incorporation of apple and strawberry powder in mutton up to a certain percentage along with the required spices, the possible outcomes that are expected are as follows: improvement in texture, taste, overall acceptability, lowering of lipid oxidation, radical scavenging activity of antioxidants, leading to a healthy edible meat and increase in dietary fibre.

Materials and Methods

The study has been conducted in the laboratories of the Department of Food Process Engineering, SRM University, Katankulathur, TamilNadu.

Raw Materials

California apples (*Malus domestica*) and strawberries (*Fragaria ananassa*) were purchased from the supermarket. The apples were scraped with the use of a knife to remove the wax coating and washed thoroughly with water. The apples were peeled using the peeler and separated from the pulp. The pulp was washed with water and mashed in the mixer. The strawberry was also mashed in the mixer. They were dried in freeze drier at the temperature of -50ºC in the presence of nitrogen for 24 hours.

The dried samples were then powdered in the mixer grinder and packed in LDPE (Low-Density Poly Ethylene) and stored at 3°C.

Boneless goat meat was purchased from the market to prepare mutton patty. Mutton masala powder, salt, ginger-garlic paste and oil were purchased from the market. The meat was cooked to be softened. Then, it was ground and minced.

Experimental Design

The moisture content was determined using the Hot Air Oven Method (AOAC 2002). The antioxidants were extracted from the fruits by Soxhlet extractor using ethanol as solvent. Total phenolic content, ferric reducing antioxidant power assay and DPPH (2,2-diphenyl-1-picrylhydrazyl) tests were conducted for the extracts.

The fruit powders were incorporated to the meat and further the proximate analysis was carried out by standard AOAC (2002) and further sensory and Standard deviation and variance of means (ANOVA) were calculated using statistical analysis.

Formulation of Mutton Patties

The ingredients for the control and the incorporated fruit powders into mutton at different concentrations are given in Table 1.

Table 1. Mutton Patty Formulation

Ingredients	Control	Concentration of fruit powder		
		3%	5%	7%
Meat	50	50	50	50
Salt	1.5	1.5	1.5	1.5
Spice mix	2.2	2.2	2.2	2.2
Oil	4.3	4.3	4.3	4.30
Ginger garlic paste	4.5	4.5	4.5	4.5
Fruit powder	0	3	5	7

Preparation of Antioxidant Solutions

Each of 2.5g of the dried fruit powder sample was taken and extracted in a Soxhlet extractor at 55°C for 8 hours using ethanol as solvent. The extract was kept at a controlled temperature and pressure and the ethanol solvent was allowed to evaporate for 24 hours. The extract was later stored at 4°C.

Preparation of Mutton Patties

The boneless meat was obtained from the market. The meat was cut into pieces and cooked in pressure cooker with salt to soften it. Once the meat was softened, it was ground in the mixer and minced. Spice mix and ginger-garlic paste along with oil was added to the minced meat and mixed thoroughly. The minced meat was divided into batches of 50g each and a part was kept as the control where no fruit powder is added. The other nine batches were assigned with the set of 3%, 5% and 7% concentrations with each of the following samples: apple peel powder, apple pulp powder and strawberry powder. The meat was shaped into patties. The different meat patties with various concentrations were packed in LDPE at 4°C for later usage.

Total Phenolic Content

The total phenolic content of mutton patties incorporated with apple peel, apple pulp and strawberries at 3%, 5% and 7% concentration were calculated using Folin-Ciocalteau method (Mohammad Amir *et al*., 2013). 0.5ml of the extract (10µg/ml) was taken and 5ml of the FC reagent (diluted to 1:10) were added. After 5 minutes, 4ml of sodium carbonate were added. It is left undisturbed for 30 minutes. Different concentrations of gallic acid were used to plot the standard graph. The absorbance was checked using UV spectrophotometer at 765nm. The amount of total phenolic content was calculated as Gallic Acid Equivalents (GAE) mg/g.

Radical Scavenging Activity using DPPH Assay

The radical scavenging activity of the extracts was determined using DPPH assay (Chang *et al*., 2007). 3 ml of 2, 2-diphenyl-1-picrylhydrazyl were mixed in 0.1mM methanol. 100 µg/ml of each of the sample extract were added to different test tubes containing methanolic DPPH solution. It was kept in the dark room for 20 minutes. The absorbance was calculated at 517nm. Radical scavenging activity of the extract was further calculated using the following equation:

$$\%\ Radicalscavengingactivity = \frac{\text{Absorbance control} - \text{absorbance sample X 100}}{\text{Absorbance control}} \quad (1)$$

Ferric Reducing Antioxidant Power (FRAP)

The ferric reducing power of the extracts was examined by the standard method (Verma *et al*., 2013). Each of the fruit extracts was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% (w/v) potassium ferricyanide in 10 ml test tubes. The mixtures were incubated for 30 mins at room temperature. Then 2.5 ml of 10% trichloroacetic acid were added and then centrifuged at 700 rpm for 10 mins. The supernatant was mixed with 2.5 ml distilled water and 0.5 ml of ferric chloride (0.1% w/v). The absorbance was measured at 700 nm. An increase in absorbance of the reaction mixture indicated the reducing power of the sample.

Peroxide Value Analysis

According to the method described by Qin *et al*. (2013), peroxide values for all the samples were analysed. 1.0 ml of fat was extracted from Soxhlet extractor. 30 ml of acetic acid-chloroform (1:3) were added and mixed thoroughly. Potassium iodide (0.5 ml) was added along with 30 ml of distilled water. A few drops of starch solution were added as the indicator. The peroxide value (PV) was evaluated by measuring the iodine released from potassium iodide titrated with standardised 0.01N sodium thiosulfate solution. The end point was checked when the blue colour disappeared.

Microbiological Analysis

To determine the bacterial count for each sample, mutton patty sample (10 g) incorporated with three different concentrations of fruit powders was aseptically transferred into a sterile stomacher bag and diluted with 90 mL, 0.1% sterile peptone water. The sample was then mixed thoroughly using the shaker. Five-fold serial dilutions (using 0.1% sterile peptone water) were made. 0.1 mL aliquot of each dilution was plated onto standard plate count agar (PCA). The plates were incubated

at 30±1°C for 48 h to determine the standard plate count after 0, 3, 6, 9, and 12 days of storage. Results were expressed as log colony forming units (CFU)/g mutton meat.

Dietary Fibre Analysis

The dietary fibre in mutton patties was determined as per the method of AOAC (1990). 3 mL of acetic/nitric reagent were added to a known amount (0.5 g or 1 g) of the sample in a test tube and mixed in a vortex mixer. The tube was placed in a water-bath at 100°C for 30 minutes. It was cooled and then centrifuged for 15–20 min. The supernatant was discarded. The residue was washed with distilled water.10 mL of 67% sulphuric acid were added and allowed to stand for 1 h. 1 mL of the above solution was diluted to 100 mL. To 1 mL of this diluted solution, 10 mL of anthrone reagent were added and mixed well. The tubes were heated in a boiling water-bath for 10 min and after cooling the colour was measured at 630 nm. A blank was set with anthrone reagent and distilled water.

Colour Analysis

Surface Colour Analysis was carried out using a Hunter Colorimeter. The samples were placed in a measuring container, and the values for L* (lightness), a* (redness), and b*(yellowness) were recorded to evaluate surface colour changes of samples during storage.

Sensory Analysis

The sensory analysis of various samples was conducted for taste, aroma, texture and appearance. The sensory evaluations were conducted on a nine-point hedonic scale. The panellists were asked to rate the acceptability of the product on a scale of 9 points ranging from 9 to be "like extremely" to 1 to be "dislike extremely".

Results and discussions

Total phenolic content

Phenolic contents are important parameters to determine antioxidant and radical scavenging activity. The test results of different fruit powders are represented in Figure 1. The highest amount of polyphenol content was seen in apple peel with 40mg/g. This could be due to high level of gallic acid, catechins and flavonols. The total phenolic content in strawberry was found to be 31mg/g and in apple pulp, it was 19 mg/g.

According to Buricova *et al*. (2011) and Shoji *et al*. (2004), the total phenolic content found in strawberry and apples falls in the range of the test conducted. The total phenolic content was also assessed for mutton patties incorporated with the fruit powders and the results are tabulated in table 2 at 3%,5% and 7% concentration. The total phenolic content on storage is found to have a decrease which may be caused by the reduction of phenolics from the fruit powders or mutton patties due to storage conditions. The meat samples incorporated with 5% apple peel found to have higher phenolic content with a significance of ($p<0.005$) compared to others.

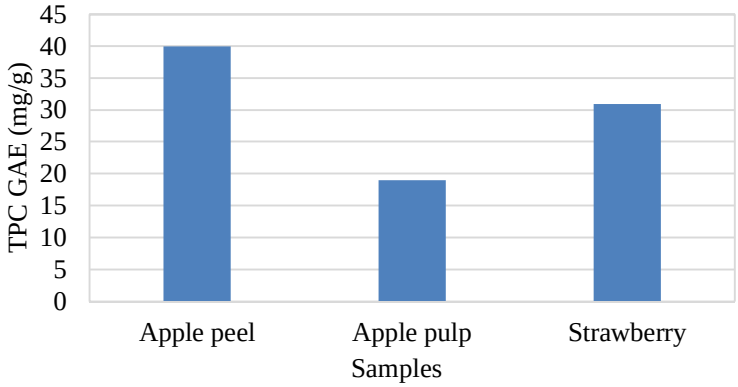

Figure 1. Total phenolic test content GAE (mg/g)

Table 1.Total phenolic content for mutton patties with fruit powder

Days	Apple peel 3%	Apple peel 5%	Apple peel 7%	Apple pulp 3%	Apple pulp 5%	Apple pulp 7%	S 3%	S 5%	S 7%
0	0.44± 0.24	0.46± 0.75	0.44± 0.24	0.29± 0.81	0.31± 0.94	0.32± 0.81	0.41± 0.80	0.42± 0.99	0.44± 0.97
3	0.41± 0.42	0.44± 0.83	0.41± 0.33	0.27± 0.24	0.28± 0.80	0.03± 0.23	0.39± 0.50	0.39± 0.86	0.41± 0.42
6	0.38± 0.98	0.41± 0.94	0.38± 0.41	0.25± 0.68	0.27± 0.33	0.29± 0.42	0.38± 0.76	0.39± 0.16	0.39± 0.10
9	0.37± 0.14	0.39± 0.64	0.38± 0.36	0.24± 0.67	0.25± 0.34	0.27± 0.34	0.38± 0.52	0.37± 0.54	0.38± 0.61
12	0.35± 0.93	0.37± 0.91	0.36± 0.45	0.24± 0.96	0.25± 0.54	0.27± 0.66	0.36± 0.89	0.36± 0.10	0.38± 0.03

s – Strawberry

Radical scavenging analysis using DPPH assay

The DPPH assay has been widely used to determine the free radical scavenging activity of various plants and pure compounds. Free radicals are present in the environment which causes cell damage or mutation leading to cancer, heart diseases and many others. Apples and strawberries are rich in ascorbic acid with high nutritional value. When an antioxidant scavenges the free radical by hydrogen donation, the colour from the DPPH assay solution becomes light yellow. The R^2 value of standard Gallic acid was found to be 0.99. The results indicate strong correlation with the R^2 value of apple peel value to be 0.946, strawberry 0.886 and apple pulp 0.816. The EC50 value for DPPH radicals were 4.2, 11.6 and 27.7mg/ml for apple peel, strawberry and apple pulp, respectively, which determines the amount of polyphenols required to scavenge 50% of the radicals. The least amount of apple peel was enough for radical scavenging effects. This result showed that DPPH radical can be scavenged by carotenoids which are highly found in apple peel. According to the test results as in table 3, in the meat samples incorporated with fruit

powder at three concentrations, the apple powder at 5% showed highly significant value of ($p<0.5$) when compared to other concentration of fruit powders. Results obtained in our study showed an increase in IC_{50} values after 12 days of storage in comparison with the starting values, which means that the total antioxidant capacity decreased. The values were in accordance with the studies conducted by Wang *et al*. (2007) and Giomaro *et al*. (2014). Figure 2 and Table 3 show the comparison values of EC50 for every sample.

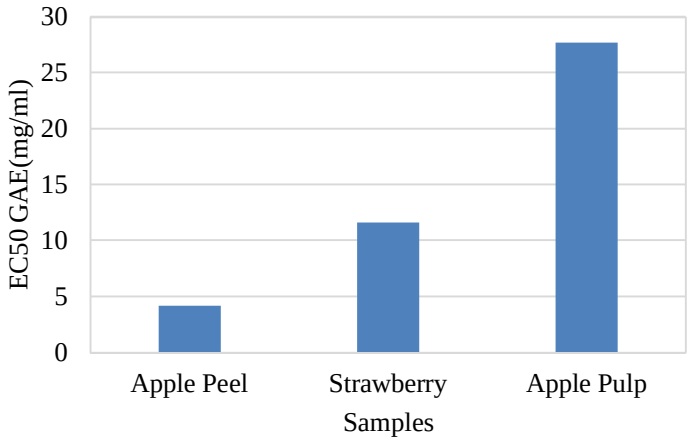

Figure 2. EC 50 values of apple peel, apple pulp and strawberry

Table 3. IC 50 value for mutton patties with fruit powder

Days	Apple peel 3%	Apple peel 5%	Apple peel 7%	Apple pulp 3%	Apple pulp 5%	Apple pulp 7%	S 3%	S 5%	S 7%
0	2.68± 0.12	2.51± 0.41	2.62± 0.45	10.48 ±0.03	10.51 ±0.35	10.54 ±0.24	7.53± 0.97	7.61± 0.71	7.64± 0.81
3	2.75± 0.08	2.72± 0.11	2.14± 0.33	10.67 ±0.05	10.75 ±0.11	10.86 ±0.28	7.62± 0.67	7.82± 0.62	7.81± 0.24
6	3.11± 0.13	2.95± 0.5	2.89± 0.38	10.91 ±0.09	11.03 ±0.89	11.12 ±0.32	7.95± 0.83	7.94± 0.95	7.89± 0.34
9	3.24± 0.09	3.11± 0.31	3.51± 0.21	11.01 ±0.15	11.11 ±0.15	11.25 ±0.43	8.05± 28	8.07± 0.82	8.11± 0.48
12	3.22± 0.08	3.34± 0.4	3.54± 0.51	11.02 ±0.25	11.15 ±0.14	11.26 ±0.27	8.1± 0.63	8.13± 0.67	8.15± 0.22

S - Strawberry

Ferric reducing antioxidant power

The antioxidant power can also be detected by the ability of the sample to reduce Fe^{3+} to Fe^{2+}. BHT served as the control. The reducing power of a compound is related to its electron transfer ability; therefore, the reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The reducing

power of apple peel and strawberry extracts increased with the increasing concentration. Apple peel extract showed higher reducing activities compared to apple pulp extract at all the described concentrations. No significant difference was observed between the reducing power of apple peel and strawberry extracts with BHT. But there was a reduction in the value of the apple pulp. Figure 3 demonstrates the results obtained.

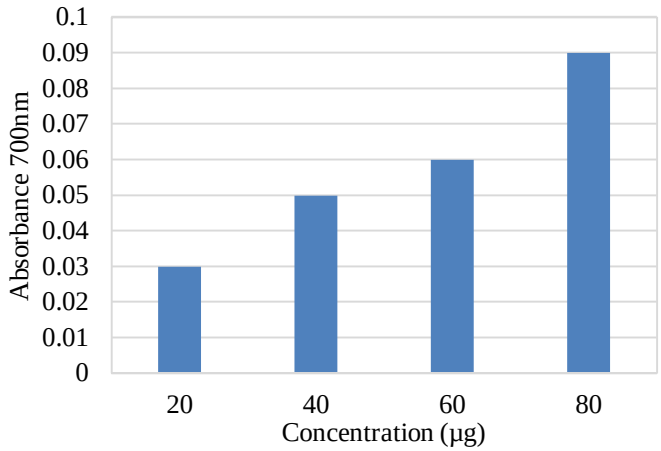

Figure 3. Concentration apple peel extract (µg) vs absorbance

Peroxide value analysis

Samples with antioxidants had reduced peroxide formation compared to that of the control group. This could be due to the antioxidant properties capable of retarding lipid oxidation. But peroxide value increased due to the formation of hydroperoxides. Meat samples incorporated with apple peel, strawberry and BHT could reduce the formation of peroxide much better than the sample incorporated with apple pulp powder as tabulated in Table 4. The result indicates that the presence of fruit powder in mutton meat patties was able to retard the formation of peroxides in the lipid.

Microbiological analysis

The result of microbiological analysis of mutton meat using different fruit powder during 12 days storage period was presented in Table5. On the basis of the results, it can be concluded that antimicrobial activity of the added fruit powder was related to the presence of phenolic compounds which were likely to be responsible for antibacterial activity. The standard plate count increased throughout 12 days of storage. The results are depicted in Table 3 as CFU/g.

Dietary fibre estimation

The sample incorporated with apple peel had a high content of dietary fibre with an average value of 3 different concentrations to be 8.1-9.1%. Sample incorporated with strawberry powder had dietary fibre in the range of 4.11-5.2% and the sample containing apple pulp had the least value with 1.5-2.47% as shown in Figure 4.

Table 4. Peroxide Value Analysis of Mutton Patties

Days	Control	Apple peel 3%	Apple peel 5%	Apple peel 7%	Apple pulp 3%	Apple pulp 5%	Apple pulp 7%	S 3%	S 5%	S 7%
0	3.3± 0.1	3.0± 0.02	2.9± 0.3	2.7± 0.1	3.2± 0.01	3.4± 0.3	3.1± 0.1	3.1± 0.05	3.0± 0.4	2.9± 0.1
3	4.1± 0.2	3.9± 0.2	3.6± 0.1	3.4± 0.01	4.1± 0.2	4.0± 0.02	4.2± 0.2	4.0± 0.09	3.9± 0.03	3.5± 0.02
6	6.2± 0.2	4.8± 0.1	5± 0.01	4.9± 0.04	6.0± 0.5	6.1± 0.5	6.0± 0.03	5.8± 0.5	4.8± 0.5	4.9± 0.5
9	7.6± 0.1	6.1±.3	6.1±0.2	6.0± 0.4	7.5± 0.04	7.5± 0.02	7.5± 0.01	6.9± 0.3	6.1± 0.5	6.0± 0.05
12	8.9± 0.03	7.3± 0.1	6.9± 0.1	6.4± 0.2	8.4± 0.4	8.3± 0.02	8.2± 0.5	8.0± 0.4	7.8± 0.1	7.0± 0.3

S - Strawberry

Table 5. Microbiological analysis of Mutton patties incorporated with fruit powder

Days	Control	Apple peel 3%	Apple peel 5%	Apple peel 7%	Apple pulp 3%	Apple pulp 5%	Apple pulp 7%	S 3%	S 5%	S 7%
0	$2.3\cdot 10^4$	$2.0\cdot 10^4$	$1.9\cdot 10^4$	$1.8\cdot 10^4$	$2.2\cdot 10^4$	$2.2\cdot 10^4$	$2.0\cdot 10^4$	$2.3\cdot 10^4$	$2.1\cdot 10^4$	$2.0\cdot 10^4$
3	$3.61\cdot 10^4$	$3.4\cdot 10^4$	$2.6\cdot 10^4$	$2.2\cdot 10^4$	$3.5\cdot 10^4$	$3.45\cdot 10^4$	$2.9\cdot 10^4$	$3.4\cdot 10^4$	$2.9\cdot 10^4$	$2.7\cdot 10^4$
6	$4.2\cdot 10^4$	$4.0\cdot 10^4$	$3.7\cdot 10^4$	$3.1\cdot 10^4$	$4.1\cdot 10^4$	$4.1\cdot 10^4$	$3.9\cdot 10^4$	$4.0\cdot 10^4$	$3.8\cdot 10^4$	$3.8\cdot 10^4$
9	$5.6\cdot 10^4$	$5.1\cdot 10^4$	$4.9\cdot 10^4$	$4.2\cdot 10^4$	$5.5\cdot 10^4$	$5.3\cdot 10^4$	$5.1\cdot 10^4$	$5.0\cdot 10^4$	$5.1\cdot 10^4$	$5.0\cdot 10^4$
12	$6.9\cdot 10^4$	$6.1\cdot 10^4$	$5.8\cdot 10^4$	$5.1\cdot 10^4$	$6.8\cdot 10^4$	$6.4\cdot 10^4$	$6.0\cdot 10^4$	$6.0\ 10^4$	$5.9\cdot 10^4$	$5.7\cdot 10^4$

S - Strawberry

Sensory analysis

A panel of 10 members analysed the stored and fresh meat samples. It was found that overall acceptability was for the meat sample incorporated with apple peel at 5% with overall acceptability of 8. There was no much difference between the acceptability rate of meat samples combined with apple pulp and strawberry powder at 3% whose acceptability was 7.

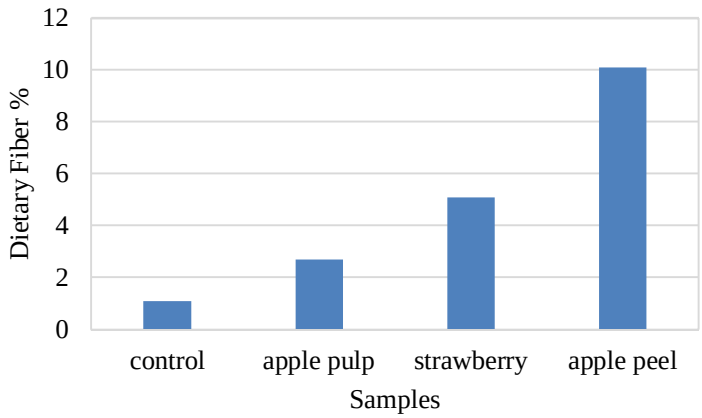

Figure 4. Dietary fibre content in meat samples with fruit incorporation

Colour analysis

The Colour index of the mutton patties incorporated with fruit powders was measured using Color Quest XE Hunter Colour Meter based on L*, a*, b* color system. 5g of the sample were taken for colour analysis. The positive values of a* and b* showed that the samples were inclined towards red and yellow colour. Incorporation of apple peel and pulp improved the yellowness and strawberry improved the redness value of the product. No significant difference was observed in lightness during the storage period. The colour values of red and yellow subsequently decreased after the storage period, as shown in Table 6.

Table 6. Colour Analysis of Mutton Patties incorporated with fruit powders

Day 15 parameters	Control	Apple peel 3%	Apple peel 5%	Apple peel 7%	Apple pulp 3%	Apple pulp 5%	Apple pulp 7%	S 3%	S 5%	S 7%
Lightness	54.6± 0.1	46.2± 0.22	47.92 ±0.39	461.7 ±0.68	51.7± 0.5	52.1± 0.1	51.9± 0.6	53.06 ±0.01	53.96 ±0.23	53.5± 0.2
Redness	4.09± 0.23	4.92± 0.19	4.83± 0.51	4.84± 0.51	4.14± 0.66	4.25± 0.02	4.25± 0.01	5.35± 0.19	5.37± 0.03	5.39± 0.22
Yellowness	28.39 ±0.2	27.11 ±0.6	26.5± 0.11	26.9± 0.44	31.1± 0.35	32.0± 0.05	33.0± 0.15	30.9± 0.51	31±0.5	30.7± 0.25

Conclusion

As per the sensory analysis, apple peel with 5% incorporation was found to have higher acceptance value with high significance of $p<0.5$. Moreover, the dietary fibre was high in apple peel as compared to the other fruit powders. It showed that it had high antioxidant value almost equal to the strawberry powder with 7% incorporation. But the taste of strawberry powder was not acceptable in mutton patties and was highly acidic. The total phenolic content was also high in apple peel. Hence, it can be concluded that apple peel incorporated in meat had better storage value and did

not affect the product's organoleptic characteristics. Therefore, apple peel at 5% incorporation can be used as a source of antioxidant and dietary fibre in mutton patties and its application will be very valuable and desirable.

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