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RESEARCH ARTICLE

Anti-oxidant activity of functional yoghurt incorporated with *Hibiscus rosa sinensis* flower extract

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Abstract: Functional herbal yoghurt was developed by incorporating *Hibiscus rosa sinensis* flower extract at one and two per cent levels. The developed yoghurt was evaluated for physico - chemical parameters, anti-oxidant activity and sensory qualities. Incorporation of hibiscus has significantly increased the anti-oxidant activity. The antioxidant activity of control yoghurt and yoghurt incorporated with one and two per cent hibiscus flower extract were 14.4±0.66, 19.78±0.66 and 36.32±0.66 per cent respectively. The titratable acidity increased with respect to the increase in concentration of hibiscus extract and period of storage. There was a significant increase in total solids content in yoghurt incorporated with two per cent hibiscus extract. Hibiscus incorporation had also increased the syneresis percentage of yoghurt. Calcium and Iron content of yoghurt increased significantly in hibiscus incorporated yoghurt. Control yoghurt had 88.97±1.56 and 1.14 ±0.01 mg/100 g of calcium and of iron respectively. Yoghurt incorporated with one and two per cent hibiscus extract had 124.88±1.67 and 224.8±1.93 mg/100 g of calcium and 1.43 ±0.01 and 1.92 ±0.01mg/100 g of iron respectively. No significant difference was observed in the fat percentage of yoghurt incorporated with hibiscus. Sensory quality of yoghurt was not altered significantly due to the incorporation of hibiscus flower extract. The study revealed that hibiscus flower extract can be incorporated into yoghurt to improve the anti- oxidant property, calcium and iron content of yoghurt without affecting the sensory qualities.

Keywords: Hibiscus, herbal yoghurt, anti-oxidant activity, calcium enriched yoghurt, functional yoghurt

Introduction

Fermented dairy products are well known for their health benefits and are consumed traditionally from time immemorial. Yoghurt is the most popular fermented dairy product throughout the world. A significant growth in the consumption of yoghurt has been reported in many countries due to its health promoting properties. Functional additives such as plant extracts, probiotics and prebiotics are nowadays incorporated into yoghurt to improve the health benefits.

Hibiscus rosa sinensis is a shrub that belongs to Malvaceae family. It is commonly known as China rose or Shoe-flower. It is considered to be a medicinal herb due to the fact that it possesses anti-oxidant, anti-tumor, anti-inflammatory and anti-microbial activity. Petals of hibiscus flowers are edible, non-toxic and contain valuable nutrients. Several studies have proved the presence of anti-oxidant, anti-fungal and antimicrobial properties in flowers of *Hibiscus* (Khan et al.. 2017 and Singh et al.. 2019)

It is important to prevent the peroxidation of lipids and formation of free radicals in order to increase the shelf life and to preserve the quality characteristics of fat rich dairy products. Lipid oxidation is inhibited by anti-oxidant agents. Anti-oxidants are compounds that can stop or limit free radicals. Synthetic anti-oxidants such as Butylated hydroxy anisole, Butylated hydroxy toluene and Propyl gallate exhibit strong anti-oxidant activity against several oxidation systems. However, the use of synthetic anti-oxidants in foodstuffs is restricted because of the potential risks to human health (Jeong et al.. 2005). Currently anti-oxidants from natural sources are receiving increased attention. Medicinal plants rich in natural anti-oxidants are increasingly used in dairy foods to improve nutritional and therapeutic properties. The

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extract of hibiscus flower contains bioflavonoids, tannins and anthocyanins responsible for the anti-oxidant property (Rengarajan et al.. 2020). The research work was designed to develop functional yoghurt incorporated with *Hibiscus rosa sinensis* flower extract and to determine the antioxidant activity and physico- chemical properties of functional yoghurt.

Material and Methods

Preparation of the aqueous extract of *Hibiscus rosa-sinensis* flower

Fresh *Hibiscus rosa sinensis* flowers were collected from Mannuthy. The petals were dried at 30ºC in a hot air oven. Powdered form of *Hibiscus rosa sinensis* flowers (5 g) were extracted under reflux condenser with sterile water (350 ml) by using accelerated solvent extractor at 100°C for 30 to 40 minutes as per the procedure described by Iwalokun (2007). Hibiscus flower extract was lyophilized in a freeze drier (Operon, -70°C). Lyophilized extract was stored in refrigerated temperature for further use.

Preparation of yoghurt

Yoghurt was prepared as per Tamime and Robinson (2007). Skim milk was standardized to 0.5 per cent fat and skim milk powder (3 per cent) was added to increase the solids-not-fat content. Then it was heated at 90°C for 10 minutes and six per cent sugar was added. The contents were mixed properly and cooled to 42°C. Two percent yoghurt culture (NCDC-145) obtained from National Collection of Dairy Cultures, NDRI, Karnal was added. Two experimental yoghurt samples were prepared by adding one and two per cent of aqueous extract of *Hibiscus rosa sinensis.*

Physico- Chemical analysis of functional yoghurt

Titratable acidity of yoghurt samples was determined according to the procedure laid out in the FSSAI (2015). The fat content of yoghurt was determined by the Rose Gottlieb method outlined by FSSAI (2015). Total solids content of yoghurt was determined by the procedure prescribed by Bureau of Indian Standards (IS: 12333, 1997). The syneresis percentage was determined as per the procedure prescribed by Doleyres and Lacroix (2005). Syneresis is expressed as percentage weight of drained whey over the initial weight of the yoghurt sample.

Anti-oxidant activity of yoghurt samples was determined as the ability of each extract to scavenge 1,1 – diphenyl 2 – picrylhydrazyl (DPPH) radicals by the procedure laid out by Rasdhari et al.. (2008). Anti-oxidant activity of yoghurt is expressed as inhibition percentage of DPPH free radicals.

Calcium and iron content of hibiscus incorporated yoghurt was determined by the procedure laid by Arslaner et al.. (2021) with some modifications. The yoghurt samples were acid digested in microwave digester by taking 2 grams of yoghurt sample in a digestion vessel. Then eight ml of 69% HNO_3 solution was added and then kept for digestion at 200°C and a pressure of 30 bar. After digestion, the vessels were allowed to cool to room temperature.

Atomic absorption spectroscopy

A hollow cathode lamp with the cathode made of calcium and iron were used as a source of light at 422.8 nm and 248.33 nm respectively. Samples were placed one by one for the mineral analysis. Two percentage HNO_3 was used as the blank solution

Sensory evaluation

Sensory evaluation of yoghurt samples was carried out by six expert panelists. Yoghurt samples were evaluated for their sensory characteristics such as color and appearance, body and texture, flavor and overall acceptability as per the score card suggested by IDF (1987).

Results and Discussion

Titratable Acidity

The mean titratable acidity values of control yoghurt (C) were 0.805 ±0.02, 0.91±0.01, 1.032±0.01 and 1.082 ±0.13 per cent lactic acid respectively on the first, third, fifth and seventh days of storage. The corresponding values of yoghurt incorporated with one per cent hibiscus flower extract (T1) were 0.798 ±0.02, 0.977 ±0.01, 1.08 ±0.01 and 1.127 ±0.13 per cent lactic acid respectively (Table 2). The mean values for yoghurt with two per cent hibiscus flower extract (T2) were 0.828±0.02, 1.03±0.01, 1.103 ±0.01and 1.148±0.13 per cent lactic acid respectively. There was a significant increase ($p<0.05$) in titratable acidity from the first day to the seventh day of storage. Higher titratable acidity values were observed in yoghurt samples incorporated with two per cent hibiscus extract (T2) and it was significantly higher than that of control and yoghurt incorporated with one percent hibiscus flower extract (T1). Kim et al. (2019) reported that the titratable acidity of yoghurt incorporated with one per cent *Hibiscus sabdariffa* extract reached up to 1.0 percent lactic acid. The results obtained are in agreement with the above findings.

Total Solids Content

The mean total solids per cent of control yoghurt (C) were 13.477±0.007, 13.608±0.004, 13.832±0.008 and 13.938±0.008 per cent respectively on the first, third, fifth and seventh day of storage. The respective values for yoghurt incorporated with one percent hibiscus flower extract (T1) were 13.408±0.007, 13.423±0.004, 13.608±0.008 and 13.823±0.008 per cent. The total solids content of yoghurt added with two per cent hibiscus flower extract (T2) were 13.463±0.007, 13.618±0.004, 14.117±0.008 and 14.133±0.008 per cent respectively. A significant increase ($p<0.05$)

in total solids content during storage was noticed. Highest total solid content was observed in yoghurt incorporated with two per cent hibiscus extract (T2) and it was significantly higher than that of control and yoghurt incorporated with one per cent extract (T1). Similar findings were reported by Arslaner et al. (2021)

Syneresis Percentage

The mean syneresis values of control yoghurt (C) were 2.18 ±0.06, 2.05 ±0.03, 2.03 ±0.02 and 2±0.03 per cent respectively on the first, third, fifth and seventh day of storage. The syneresis values of yoghurt incorporated with one per cent hibiscus flower extract (T1) were 3.53 ±0.06, 2.42 ±0.03, 2.47 ±0.02 and 2.3 ±0.03 per cent respectively. The corresponding values for yoghurt with two per cent hibiscus flower extract (T2) were 3.08 ±0.06, 2.43 ±0.03, 2.45 ±0.02 and 2.37 ±0.03 per cent respectively. A significant difference in the mean syneresis value was noticed in all yoghurt samples ($p<0.05$) during storage. Highest syneresis value was observed in yoghurt sample incorporated with one per cent of hibiscus extract (T1) and it was significantly different from the yoghurt incorporated with two per cent extract (T2) and that of control. Nguyen (2021) found that yoghurt incorporated with *Hibiscus sabdariffa* had higher syneresis value than control.

Fat Percentage

The mean fat percentage of control yoghurt were 0.17 ±0.03, 0.2 ±0.03, 0.2 ±0.03 and 0.2 ±0.03 per cent respectively on the first, third, fifth and seventh day of storage. The fat percentage of yoghurt incorporated with one per cent hibiscus flower extract (T1) were 0.23 ±0.03, 0.22 ±0.03, 0.22 ±0.03 and 0.22 ±0.03 per cent. The respective values for yoghurt prepared with two per cent hibiscus flower extract (T2) were 0.2 ±0.03, 0.2 ±0.03, 0.22 ±0.03 0.22 ±0.03 and 0.2 ±0.03 per cent. No significant difference in fat percentage was observed between yoghurt samples. Kavas et al.. (2003) had also reported no appreciable change in the fat content of yoghurt incorporated with hibiscus.

Antioxidant Activity

The mean antioxidant activities of control yoghurt (C) were 10.73±0.71, 14.4±0.66, 8.38±0.46 and 7.24±0.46 per cent respectively on the first, third, fifth and seventh day of storage. The mean values for yoghurt incorporated with one per cent hibiscus flower extract (T1) were 17.76±0.71, 19.78±0.66, 11.85±0.46 and 10.71±0.46 per cent respectively. The corresponding mean values obtained for yoghurt with two percent hibiscus flower

Table 1: Antioxidant activity (Mean ± S.E) of yoghurt (percentage)

Sample	1st day	3rd day	5th day	7th day	Overall
C	10.73 ± 0.71^{Bc}	14.4 ± 0.66^{aC}	8.38 ± 0.46^{cC}	7.24 ± 0.46^{cC}	10.19 ± 0.31^{C}
T1	17.76 ± 0.71^{bB}	19.78 ± 0.66^{aB}	11.85 ± 0.46^{cB}	10.71 ± 0.46^{cB}	15.02 ± 0.31^{B}
T2	25.5 ± 0.71^{bA}	36.32 ± 0.66^{aA}	19.71 ± 0.46^{cA}	17.73 ± 0.46^{dA}	24.82 ± 0.31^{A}
Overall	18 ± 0.41^{b}	23.5 ± 0.38^{a}	13.31 ± 0.26^{c}	11.89 ± 0.26^{d}	

Table 2: Physico-chemical parameters of yoghurt (Mean ± S.E)

Parameters	Samples	Storage Days			
		1st Day	3rd Day	5th Day	7th Day
Titratable acidity (in per cent lactic acid)	C	0.805 ± 0.02^{dB}	0.91 ± 0.01^{cC}	1.032 ± 0.01^{bC}	1.082 ± 0.13^{aC}
	T1	0.798 ± 0.02^{dB}	0.977 ± 0.01^{cB}	1.08 ± 0.01^{bB}	1.127 ± 0.13^{aB}
	T2	0.828 ± 0.02^{dA}	1.03 ± 0.01^{cA}	1.103 ± 0.01^{bA}	1.148 ± 0.13^{aA}
Total solids content (%)	C	13.477 ± 0.007^{Da}	13.608 ± 0.004^{cA}	13.832 ± 0.008^{bC}	13.938 ± 0.008^{aB}
	T1	13.408 ± 0.007^{Cb}	13.423 ± 0.004^{cB}	13.608 ± 0.008^{aB}	13.823 ± 0.008^{bC}
	T2	13.463 ± 0.007^{cA}	13.618 ± 0.004^{bA}	14.117 ± 0.008^{aA}	14.133 ± 0.008^{aA}
Syneresis (%)	C	2.18 ± 0.06^{aB}	2.05 ± 0.03^{abB}	2.03 ± 0.02^{bB}	2 ± 0.03^{Bb}
	T1	3.53 ± 0.06^{aA}	2.42 ± 0.03^{bA}	2.47 ± 0.02^{bA}	2.3 ± 0.03^{cA}
	T2	3.08 ± 0.06^{aC}	2.43 ± 0.03^{bA}	2.45 ± 0.02^{bA}	2.37 ± 0.0^{bA}
Fat (%)	C	0.17 ±0.03	0.2 ±0.03	0.2 ±0.03	0.2 ±0.03
	T1	0.23 ±0.03	0.22 ±0.03	0.22 ±0.03	0.22 ±0.03
	T2	0.20 ±0.03	0.20 ±0.03	0.22 ±0.03	0.22 ±0.03

Table 3: Calcium and Iron content (Mean ± S.E) mg/100g of yoghurt

Sample	Calcium	Iron
C	88.97 ± 1.56^{C}	1.14 ± 0.01^{C}
T1	124.88 ± 1.67^{B}	1.43 ± 0.01^{B}
T2	224.8 ± 1.93^{A}	1.92 ± 0.01^{A}

Table 4: Sensory scores (Mean ± S.E) of yoghurt

Sample	Appearance and colour	Body and texture	Flavor	Overall acceptance
C	4.44±0.29	4.56±0.24	9.34±0.17^{A}	18.34±0.8
T1	4.56±0.18	4.66±17	9.34±0.17^{A}	18.56±0.52
T2	4.44±0.24	4.44±0.24	8.22±0.35^{B}	17.11±1.16

extract (T2) were 25.5±0.71, 36.32±0.66, 19.71±0.46 and 17.73±0.46 per cent (Table 1). A significant decrease in the antioxidant activity from the third day was noticed in all yoghurt samples ($p<0.05$). Higher antioxidant activity was observed in yoghurt containing two percent hibiscus flower extract (T2) and it was significantly different from that of control and from yoghurt incorporated with one per cent hibiscus flower extract (T1). Higher anti-oxidant activity in hibiscus incorporated yoghurt was also reported by Biomy et al. (2017) and Hamwenye et al. (2020).

Calcium and iron content

The mean calcium content of control yoghurt (C) was 88.97±1.56 mg/100g. The corresponding values of yoghurt prepared with one and two per cent hibiscus flower extract were 124.88±1.67 and 224.8±1.93 mg/100g respectively The mean iron content of control yoghurt (C) was 1.14 ±0.01 mg/100g. The corresponding values for yoghurt incorporated with one and two percent hibiscus flower extract (T1) were 1.43 ±0.01 and 1.92±0.01 mg/ 100g respectively (Table 3). A significant increase in mean calcium and iron content was noticed in treatment groups of yoghurt samples ($p<0.05$). Highest value was observed in yoghurt containing two percent hibiscus flower extract (T2) and it was significantly higher than that of control and yoghurt incorporated with one per cent hibiscus flower extract (T1). Similar findings were reported by Bahuguna et al. (2018).

Sensory Evaluation

The mean values of appearance and color, body and texture, flavor and overall acceptability score of control yoghurt (C) were 4.44±0.29, 4.56±0.24, 9.34±0.17 and 18.34±0.8 respectively. The respective values for yoghurt incorporated with one percent hibiscus flower extract (T1) were 4.56±0.18, 4.66±17, 9.34±0.17 and 18.56±0.52 (Table 4). The values for yoghurt incorporated with two percent hibiscus flower extract (T2) were 4.44±0.24, 4.44±0.24, 8.22±0.35 and 17.11±1.16 respectively. A significant decrease in flavor score was observed in yoghurt sample incorporated with two per cent hibiscus extract when compared to control yoghurt. However, no significant difference was observed in the overall acceptance of yoghurt samples. Tomar et al. (2020) had also reported positive effect of hibiscus on sensory and functional properties of yoghurt.

Conclusion

In the present study yoghurt incorporated with *Hibiscus rosa sinensis* flower extract showed higher antioxidant activity than the control yoghurt. The incorporation of hibiscus significantly increased the titratable acidity, total solids, syneresis, calcium and iron content. No significant difference was observed in the fat percentage. Sensory quality of yoghurt was not altered significantly by the incorporation of hibiscus. The study revealed that hibiscus flower extract can be incorporated into yoghurt to improve the antioxidant activity, calcium and iron content of yoghurt without affecting the sensory qualities

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