Full Length

ANTIOXIDANT ACTIVITY OF HERBAL YOGHURT INCORPORATED WITH TULSI (*OCIMUM SANCTUM*) EXTRACT

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ABSTRACT

The aim of the study was to develop herbal yoghurt by incorporating tulsi (Ocimum sanctum) leaf extract at 1 and 1.5 per cent level. The developed herbal yoghurt samples were analysed for physico-chemical properties, antioxidant activity and sensory quality by standard methods. The incorporation of tulsi extract improved the antioxidant activity of yoghurt significantly. The antioxidant activity of control and tulsi incorporated yoghurt (1 and 1.5 per cent) were 28.12±2.16, 41.82±1.51 and 53.1±1.51 per cent respectively. A decline in antioxidant activity was observed in all yoghurt samples after the third day of storage. No significant difference in fat and total solids content was observed in yoghurt samples incorporated with tulsi extract. The sensory scores of yoghurt incorporated with one per cent tulsi extract was significantly higher than that of control. The study revealed that tulsi extract can be incorporated in to yoghurt to enhance the antioxidant activity without compromising the sensory quality.

Key words: yoghurt, tulsi, antioxidant activity, herbal yoghurt

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INTRODUCTION

Consumption of fermented foods, especially fermented milk products, is associated with numerous health benefits. Yoghurt is the most extensively consumed fermented dairy product that has great consumer acceptability due to its positive health effects. Apart from its health benefits, it can also act as a carrier for functional ingredients. Various plant parts are added to improve the nutritional as well as functional properties of yoghurt. Extracts of herbs such as tulsi leaf (*Ocimum sanctum*), clove (*Syzygium aromaticum*), thyme (*Thymus vulgares*), rosemary (*Rosemarinus officinalis*), turmeric (*Curcuma longa*) and cinnamon bark (*Cinnamomum zeylanicum*) are incorporated into yoghurt to enhance the health promoting potential. Many of these herbs have antioxidant potential which can contribute great antioxidant activity to the product (Paswan *et al.,* 2021). Natural

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antioxidants, especially phenolics and flavonoids, present in these herbs are safe and also bioactive which are capable of absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides. Even though synthetic antioxidants are easily available and more economical they are quite unsafe and their toxicity is a major concern.

Basil is the general name for the culinary herb *Ocimum sanctum* of the family Lamiaceae (mints). Among four variations of Basil viz. *O. sanctum*, *O. americanum*, *O. basilicum* and *O. gratissimum*, the most preferred one for the development of yoghurt was *O. sanctum* (Hanumanthaiah *et al.,* 2020). Tulsi is well known for it's religious, spiritual and medicinal importance in India. The scientific truth behind the medicinal properties of basil is the presence of phyto chemicals like poly phenols, flavonoids, terpenoids, eugenol and alkaloids in its leaves which gives the ability to overcome infection (Hanumanthaiah *et al.*, 2020). The aqueous extract of *O. sanctum* leaves showed anti-bacterial, anti-oxidant, anti-stress, anti-asthmatic, anti-fungal, anti-viral and anti-mutagenic activities (Mittal *et al.,* 2020). Hence the present study is designed to develop herbal yoghurt by incorporating tulsi leaf extract.

MATERIALS AND METHODS

Preparation of aqueous extract of *Ocimum sanctum* leaves

Aqueous extract of tulsi was prepared by the procedure suggested by Shori and Baba (2011) with slight modifications. Fresh tulsi leaves were collected and washed properly and dried at room temperature away from sunlight. The dried leaves were powdered. One hundred gram of powder was then wrapped in a muslin cloth and immersed in a beaker containing autoclaved distilled water at 60°C. Beaker was then kept in a water bath maintained at 70°C till the complete extraction occurred. Then the extract was kept in a rotatory vacuum evaporator (Buchi) at 45°C under reduced pressure.

Lyophilization of tulsi extract

After the evaporation of excess water, the concentrated extract of *O. sanctum* was freeze dried in a lyophilizer (Operon, -70°C freeze drier). Lyophilized extract was then stored at refrigerated temperature for further use.

Preparation of yoghurt

Yoghurt was prepared as per the procedure of Tamime and Robinson (2007). Cow milk was used for the preparation of yoghurt. It was then heated at 90°C for 15 minutes. Sugar was added at six per cent level. Then the contents were mixed properly and cooled to 42°C. Two per cent yoghurt culture (NCDC-145) obtained from National Collection of Dairy Culture (NCDC), Karnal was added. Two experimental yoghurt samples were prepared by adding 1 and 1.5 per cent aqueous extract of *O.sanctum*. It was then incubated for 37°C for overnight.

Physico - chemical analysis of functional yoghurt.

Titratable acidity of yoghurt samples

was determined according to FSSAI (2015). The fat content of yoghurt sample was determined as per Bureau of Indian Standards (IS: 1224,1977). Total solids content of yoghurt was determined by the procedure outlined by Bureau of Indian Standards (IS: 12333, 1997). The syneresis percentage was determined as per Doleyres and Lacroix (2005). Syneresis was expressed as percentage weight of drained whey over the initial weight of the yoghurt sample.

Anti-oxidant activity of yoghurt was determined as the ability of extract to scavenge 1,1 – diphenyl 2 – picrylhydrazyl (DPPH) radicals by the procedure suggested by Debasree Ghosh (2019) with slight modifications. Anti-oxidant activity of yoghurt was expressed as inhibition percentage of DPPH free radicals.

Sensory evaluation

Sensory evaluation of yoghurt samples was carried out by faculty members and post graduate students of the Department of Dairy Science. Yoghurt samples were evaluated for their sensory characteristics such as colour and appearance, body and texture, flavour and overall acceptability as per the score card suggested by IDF (1987).

RESULTS AND DISCUSSION

Titratable acidity (percentage lactic acid) of yoghurt

The mean titratable acidity (TA) values of control yoghurt (C) were 0.85±0.03, 1.02±0.02, 1.09±0.01 and 1.17±0.03 per cent lactic acid on the first, third, fifth and seventh days of storage respectively. The respective TA values of yoghurt containing 1 per cent tulsi extract (T1) were 0.96±0.03, 1.04±0.02, 1.1±0.01 and 1.19±0.03 per cent lactic acid. The mean TA values of yoghurt containing 1.5 per cent tulsi extract (T2) were 0.96±0.03, 1.04±0.02, 1.12±0.01 and 1.21±0.03 per cent of lactic acid respectively. There was a significant ($p<0.05$) difference in TA between control and treatment groups on the first day. During storage, TA increased significantly in all groups of yoghurt samples. Higher TA values were observed in yoghurt with 1.5 per cent tulsi extract on seventh day of storage, but it was not significantly ($p>0.05$) different from that of control and T1. According to Kumari *et al.,* (2011), the average acidity of yoghurt with 0.2, 0.3 and 0.4 per cent tulsi paste were 0.19, 0.23 and 0.24 per cent lactic acid, respectively. Acidity increased with increase in concentration of tulsi extract. Amirdivani and Baba (2011) reported that titratable acidity of basil yoghurt was higher than that of plain yoghurt. Herbal sandesh incorporated with 0, 1, 2 and 3 per cent of tulsi extracts had TA values of 0.25, 0.26, 0.28 and 0.3 per cent lactic acid, respectively (Husain *et al.,* 2015). Similar results were also reported by Anand *et al.* (2018). An insignificant increase in TA with the increase in the concentration of tulsi extract observed in the present study might be due to the increase in the metabolic activity of yoghurt starter due to the components of tulsi.

Fat percentage of yoghurt

The mean fat content of control yoghurt samples were (C) 3.78±0.05, 3.73±0.02, 3.73±0.04 and 3.79±0.05 per cent during 1, 3, 5 and 7 days of storage

respectively. The respective values for yoghurt with 1 per cent tulsi leaf extract (T1) were 3.75±0.05, 3.75±0.02, 3.58±0.04 and 3.59±0.05 per cent. For yoghurt with 1.5 per cent tulsi extract (T2), the corresponding values were 3.88±0.05, 3.73±0.02, 3.63±0.04 and 3.64±0.05 per cent. There was no significant change in the fat content between control and treatment groups on the first and third day of storage. Further increase in storage period caused an insignificant ($p>0.05$) decrease in fat content. Highest fat percentage was shown by control yoghurt which was not significantly different from T1 and T2. Similarly, Trivedi *et al.* (2014) reported that, addition of basil powder resulted in a slight decrease in the fat content of the experimental ice cream when compared to the control. Results of Husain *et al.* (2015) were also in agreement with this study. An insignificant decrease in the fat content of yoghurt incorporated with tulsi extract might be due to an effect of increasing fat-free dry matter (Trivedi *et al.,* 2014).

Total solids percentage of yoghurt

The mean total solids per cent of control yoghurt (C) were 20.95±0.21, 21.19±0.21, 21.27±0.16 and 21.26±0.25 during 1, 3, 5 and 7 days of storage, respectively. The corresponding values for yoghurt incorporated with 1 per cent tulsi extract (T1) were 21.07±0.21, 21.24±0.21, 21.36±0.16 and 21.34±0.25 per cent, respectively. The mean total solids content of yoghurt samples treated with 1.5 per cent tulsi extract (T2) were 21.11±0.21, 21.34±0.21, 21.62±0.16, 21.61±0.25 per cent on the first, third, fifth and seventh days of storage, respectively. There was no significant ($p>0.05$) difference in total solids content between control and treatment groups of yoghurt. During storage there was a significant ($p<0.05$) increase in total solids content in control as well as treatment groups of yoghurt. Highest total solids content was observed in T2 but it was not significantly different from T1. Trivedi *et al.* (2014) found that addition of basil powder (0.5, one and 1.5 per cent) to ice cream samples increased the total solids, ash and total carbohydrate content significantly. Contrary, Husain *et al.* (2015) found that herbal sandesh with 0, 1, 2, or 3 percent tulsi extract showed a decrease in total solids content as the concentration of tulsi extract increased. A significant increase in total solids content was observed during storage which could be attributed to the evaporation of moisture during refrigerated storage.

Syneresis percentage

The mean syneresis values of control yoghurt (C) was 3.51±0.16, 2.85±0.22, 3.06±0.21 and 3.45±0.16 per cent, respectively during 1, 3, 5 and 7 days of storage. The values for yoghurt incorporated with 1 per cent tulsi extract (T1) were 3.45±0.16, 3.39±0.22, 3.52±0.21 and 3.78±0.16 per cent, respectively. The corresponding mean values for yoghurt with 1.5 per cent tulsi extract (T2) were 3.81±0.16, 3.82±0.22, 3.76±0.21 and 3.95±0.16 per cent, respectively. No significant ($p>0.05$) difference in mean syneresis values was observed between control and treatment groups on the first day. During storage a significant ($p<0.05$) increase in syneresis per centage was observed in treatment

groups of yoghurt samples. The highest syneresis value was observed in yoghurt with 1.5 per cent tulsi extract on seventh day of storage. Anand *et al.* (2018) observed a slight increase in the syneresis percentage after incorporating *Ocimum sanctum* essential oil in yoghurt at a concentration of 0.5 and 0.8 µL/ml. The results of Mittal *et al.* (2020) are in agreement with the above findings, in which the addition of 0.5, 1 or 1.5 percent *Ocimum sanctum* extract to fruit yoghurt resulted in an insignificant increase in syneresis percentage over the control. The increase in syneresis could be attributed to the presence of polyphenols, which might have reacted with milk proteins and caused the shrinkage of the casein gel.

Antioxidant activity of yoghurt incorporated with tulsi extract

The mean antioxidant activity of control yoghurt samples (C) during 1, 3, 5 and 7 days of storage were 24.85±1.51, 30.37±1.01, 26.02±1.65 and 23.58±1.83 per cent, respectively. The mean values of yoghurt added with 1 per cent tulsi leaf extract (T1) were 41.82±1.51, 35.56±1.01, 32.48±1.65 and 25.13±1.83 per cent, respectively. The corresponding mean values for yoghurt with 1.5 per cent tulsi extract (T2) were 53.1±1.51, 37.78±1.01, 34.32±1.65 and 32.85±1.83 per cent, respectively. Treatment groups had significantly ($p<0.05$) higher antioxidant activity than the control. The maximum antioxidant activity was observed in yoghurt with 1.5 per cent tulsi extract (T2). During storage both control and treatment groups of yoghurt samples showed a significant reduction in their antioxidant activity. Similar results were reported by Yildiz and Eyduran (2009). They have also reported decrease in antioxidant activity during refrigerated storage. Amirdivani and Baba (2011) observed a higher antioxidant activity at the end of fermentation and throughout the storage period in herbal yoghurt with basil than plain yoghurt. Cow milk curd treated with five per cent v/v *Ocimum basilicum* (tulsi) extract increased the DPPH scavenging activity to 40.32 per cent, the control had a value of 18.03 per cent (Debasree Ghosh, 2019). Mittal *et al.* (2020) observed that the antioxidant activity of fruit yoghurt was significantly increased by the increase in concentration of tulsi. A decrease in antioxidant activity of tulsi incorporated yoghurt after the seventh day of storage was reported by Tomar *et al.* (2021). The higher antioxidant activity of yoghurt might be due to the presence of polyphenol and anthocyanin constituents of tulsi and metabolic products produced by bacterial activity. The presence of radical scavenging activity in the control formulations could be attributed to bioactive peptides found in yoghurt. The reduced radical scavenging activity during storage is possibly due to increasing degradation of phenolic compounds and/or milk protein-polyphenol interaction during storage.

Sensory evaluation of yoghurt incorporated with tulsi extract

The mean sensory scores of control yoghurt (C) for appearance and colour, body and texture, flavour and overall acceptability were 4.83±0.12, 4.56±0.10, 8.89±0.59 and 18.28±0.64, respectively. The sensory scores for yoghurt with one per cent tulsi extract (T1) were 4.83±0.12, 4.56±0.10, 9.56±0.44

and 18.94±0.63, respectively. Scores for yoghurt with 1.5 per cent tulsi extract (T2) were 4.94±0.56, 4.44±0.10, 8.22±0.36 and 17.61±0.32, respectively. Control and treatment groups showed no significant ($p>0.05$) difference in appearance and colour, body and texture scores. An insignificant ($p>0.05$) difference was observed for the flavour score between control yoghurt and T2. The flavour score obtained for T1 was significantly ($p<0.05$) higher than T2 and C. The overall acceptance score of T1 was significantly higher than T2. Amirdivani and Baba (2011) reported lower sensory scores for *O. basilicum*- yoghurt in comparison to plain-yogurt. Kumari *et al.* (2011) reported that, the average ratings for taste and flavour of yoghurt with 0.2, 0.3 and 0.4 per cent tulsi were 8.57, 7.71 and 7.57 and the average scores for colour and appearance were 7.54, 7.71 and 8.71 respectively. On the basis of average flavour score and overall rating, Trivedi *et al*. (2014) observed that ice cream with 1 per cent freeze-dried basil powder was preferred the most, while the other three levels (0, 0.5 and 1.5) resulted in moderately lower values. Mittal *et al.* (2020) found that, herbal fruit yoghurt was acceptable up to one per cent of tulsi leaf extract, but sensory scores of yoghurt decreased when concentration of tulsi leaf extract was increased. Incorporation of tulsi extract at one per cent level had the highest flavour score and overall acceptance. In terms of flavour and overall acceptability, treatment group with 1.5 percent tulsi had obtained lower scores than the control group. The lower scores at higher concentrations of tulsi can be attributed to higher syneresis and strong flavour intensity at higher levels.

CONCLUSION

The present results have provided salient insight into the production of herbal yoghurt from cow milk by incorporating tulsi (*Ocimum sanctum*) extract. The incorporation of tulsi extract improved the antioxidant activity of yoghurt significantly. A decline in antioxidant activity was observed in all yoghurt samples after the third day of storage. No significant difference in fat and total solids content was observed in yoghurt samples incorporated with tulsi extract. The sensory scores of yoghurt incorporated with one per cent tulsi extract were significantly higher than that of control.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

Table.1. Physico-chemical parameters of yoghurt (Mean ± S.E)

Parameters	Samples	Storage Days			
		1st Day	3rd Day	5th Day	7th Day
Titratable acidity (% lactic acid)	Control	0.85 ± 0.03^{Aa}	1.02 ± 0.02^{b}	1.09 ± 0.01^{c}	1.17 ± 0.03^{d}
	T1 (1% tulsi)	0.96 ± 0.03^{Ba}	1.04 ± 0.02^{b}	1.1 ± 0.01^{c}	1.19 ± 0.03^{d}
	T2 (1.5% tulsi)	0.96 ± 0.03^{Ba}	1.04 ± 0.02^{b}	1.12 ± 0.01^{c}	1.21 ± 0.03^{d}
Fat (%)	Control	3.78 ± 0.05^{a}	3.75 ± 0.02^{ab}	3.73 ± 0.04^{Abc}	3.70 ± 0.05^{Ac}
	T1 (1% tulsi)	3.77 ± 0.05^{a}	3.75 ± 0.02^{a}	3.69 ± 0.04^{ABb}	3.66 ± 0.05^{ABb}
	T2 (1.5% tulsi)	3.78 ± 0.05^{a}	3.73 ± 0.02^{b}	3.65 ± 0.04^{Bc}	3.62 ± 0.05^{Bc}
Total solids content (%)	Control	20.95 ± 0.21^{a}	21.19 ± 0.21^{b}	21.27 ± 0.16^{b}	21.26 ± 0.25^{b}
	T1 (1% tulsi)	21.07 ± 0.21^{a}	21.24 ± 0.21^{b}	21.36 ± 0.16^{b}	21.34 ± 0.25^{b}
	T2 (1.5% tulsi)	21.11 ± 0.21^{a}	21.34 ± 0.21^{b}	21.62 ± 0.16^{b}	21.61 ± 0.25^{b}
Syneresis (%)	Control	3.51 ± 0.16^{a}	2.85 ± 0.22^{Ab}	3.06 ± 0.21^{Ab}	3.45 ± 0.16^{a}
	T1 (1% tulsi)	3.45±0.16	3.39 ± 0.22^{AB}	3.52 ± 0.21^{AB}	3.78±0.16
	T2 (1.5% tulsi)	3.81±0.16	3.82 ± 0.22^{B}	3.76 ± 0.21^{B}	3.95±0.16

Table.2. Antioxidant activity (Mean ± SE) in yoghurt (%)

Sample	1st day	3rd day	5th day	7th day	Overall mean
Control	24.85 ± 1.51^{Aac}	30.37 ± 1.01^{Ab}	26.02 ± 1.65^{Aa}	23.58 ± 1.83^{Ac}	26.21 ± 1.12^{A}
T1 (1% tulsi)	41.82 ± 1.51^{Ba}	35.56 ± 1.01^{Bb}	32.48 ± 1.65^{Bb}	25.13 ± 1.83^{Ac}	33.75 ± 1.12^{B}
T2 (1.5%tulsi)	53.1 ± 1.51^{Ca}	37.78 ± 1.01^{Bb}	34.32 ± 1.65^{Bbc}	32.85 ± 1.83^{Bc}	39.51 ± 1.12^{C}
Overall mean	39.92 ± 1.51^{a}	34.57 ± 1.01^{b}	30.94 ± 1.65^{c}	27.18 ± 1.83^{d}	

At 5 per cent level means with same superscripts (small letters a-d within a row, capital letters A-C within a column) do not differ significantly.

Table.3. Sensory evaluation of (Mean ±SE) yoghurt samples

Sample	Appearance and colour	Body and texture	Flavour	Overall acceptance
Control	4.83±0.12	4.56±0.10	8.89±0.59A	18.28±0.64AB
T1 (1% tulsi)	4.83±0.12	4.56±0.10	9.56±0.44B	18.94±0.63A
T2 (1.5%tulsi)	4.94±0.56	4.44±0.10	8.22±0.36A	17.61±0.32B

At 5 per cent level means with same superscripts (capital letters A-B within a column) do not differ significantly.

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