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## Studies on antioxidant properties and free fatty acid of Herbal Sandesh prepared with Ashwagandha (*Withania somnifera*) and Tulsi (*Ocimum sanctum*)

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### Abstract

Present study involved analyzing antioxidant properties and free fatty acid (FFA) of Herbal Sandesh. Sandesh was prepared under standard procedure and the above mentioned herbs were incorporated in the Herbal Sandesh @ 1%, 2%, and 3% and combination of Ashwagandha and Tulsi 2%, 3%, 4%, 5% and 6% in the form of extract in treatments. Effect of different level and herbs ratio and sensory scores for all attributes were highly acceptable for Sandesh A<sub>2</sub>B<sub>2</sub> made with 96 % Chhana, 2.0% Ashwagandha and 2.0% Tulsi extract. The products were tested to find the antioxidant properties were measured by DPPH (1, 1-diphenyl-2-picryl-hydrazyl) assay method and free fatty acid percentage (FFA) were measured by titration method. The sample (A<sub>3</sub>B<sub>3</sub>) showed promising results as the percentage of antioxidant activity was 43.80 (maximum) and the percentage of free fatty acid (% Oleic acid) was 0.31 (minimum) obtained for the formulation containing Ashwagandha 3%, Tulsi 3 % which considered as best sample Sandesh among other samples. It can be concluded that herbs possessed antioxidant properties and the levels of herbal treatment had a positive effect on antioxidant activity and they were effective in delaying oxidation in Sandesh, hence they can be used as sources of natural antioxidants.

**Keywords:** Antioxidants, Herbal Sandesh, DPPH assay, medicinal herbs, free fatty acid

### 1. Introduction

Sandesh is an indigenous milk product used as sweet dairy desserts, prepared by acid and heat coagulation of milk. It is a well-known sweet in the eastern part of India, particularly in West Bengal. It is an enriched with high quality animal protein, fat, minerals and vitamins. It is appreciated by people belonging to every social group. In dairy products, lipid and protein oxidation are the most important chemical changes causing deterioration in milk products quality during cooking and storage leading to rancidity. Natural antioxidants, with potential nutritional and therapeutic value, can be used for increasing the stability of foods by preventing lipid peroxidation [1-6]. Interest in natural sources of antioxidants like herbs and spices which are used as flavoring agents in many dishes has increased of late, and they can be used as natural food preservatives [2]. These natural preservatives are gaining importance in recent years because of little or no harmful effects. Herbal extracts with high antioxidant activity exhibited relatively high total phenolic contents [9]. Lipid oxidation causes development of off-odours and off-flavours whereas muscle pigment oxidation has been reported to negatively affect colour, appearance and acceptability of meat [8]. Application of antioxidants to fresh and processed meat prevents oxidative rancidity, delays development of off-flavours and improves colour stability in fresh and processed meat [14].

Buffalo milk Chhana leads to hard body and coarse textured sweet which is not desirable. Several varieties of Sandesh are available in the market and each varieties of Sandesh are sold in the market and each varieties differs in appearance, flavor, body and texture and composition. There are two types of Sandesh available. One a drier variety made from old chhana. This product is considered a delicacy and commands a much higher price. This is normal quality sandesh and has higher keeping quality than the second type which is softer and is more expensive. It is made from fresh chhana. Attributes of Sandesh are; Colour-creamy white, flavor-sweet, nutty flavor, body and texture-soft body with fine and uniform grains, appearance-dry and smooth, taste-sweet.

Chemical composition of Sandesh from buffalo milk, Moisture-27.14%, Fat-18.42%, Protein-18.71%, Sugar-33.83%, Ash-1.90%.

Modern medicine or allopathy is very costly and poor people cannot afford it. Now-a-days Ayurveda has become popular not only in India but also in many developing and developed countries due to the fact that there is no side effect [13]. A World Health Organization survey indicated that about 70-80% of the world's population rely on non-conventional medicines, mainly derived from herbal sources, for holistic healthcare. This is specially the case in developing countries where the cost of treatment is beyond the means of most people [12]. The advantages of certain ayurvedic herbs are well documented in literature. Low cost nutritive biscuits made of ayurvedic components shatavari, Ashwagandha and Yastimadhu powder was developed [11]. The medicinal properties of Ashwagandha have been studied well and reviewed in detail [6]. Aromatic herbs exhibit strong antioxidant and antimicrobial properties, which exceed many currently used natural and synthetic antioxidants and hence this interests both industry and research scientists alike. Some vitamins, flavonoids, terpenoids, carotenoids, phytoestrogens, minerals, etc. are the substances that impart these properties to spices and function as antioxidant components and preservative agents in foods [5]. Most of the active principles of ayurvedic and unani medicines are found in *W. somnifera* and around hundred different preparations are already commercially available [15]. The addition of tulsi paste at 0.2 per cent, 0.3 per cent and 0.4 per cent level improved the taste and flavour, colour and appearance, body and texture and also overall acceptability of herbal yoghurt. There was less number of yeast and mould and no number of coliform counts because of proper maintenance of sanitary condition. It is also due to the anti-microbial and anti-bacterial properties of herbal paste (Tulsi) added in low fat herbal yoghurt [10]. The herbal products are gradually gaining popularity in the world market due to presence of natural antioxidants and functioning active ingredients. They used Tulsifor the preparation of various traditional herbal sweets like Sandesh, Chamcham, etc. [11].

## 2. Material and Methods

The present study has been carried out in the research Lab, Warner School of Food and Dairy Technology, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad, U.P. (India). All the raw materials were collected from the local market of Allahabad. Potable water was used for preparing the product. It was ensured that the materials used were free from any kind of infection.

**2.1 Herbs:** Ashwagandha root powder and Tulsi leaves dried form were purchased from Allahabad city.

**2.2 Preparation of herbal water extract:** Herbal water extract was prepared by soaking each herb in distilled water (1:10) overnight followed by centrifugation (2000 rpm; 15 min at 40 °C). The supernatant was harvested and refrigerated and used in the preparation of Herbal Sandesh.

**2.3 Preparation of chhana:** The method adopted to prepare chhana in this study was according to the method given by Bhattacharya *et al.*, (1971)[3] with slight modification. The standardized buffalo milk was heated up to 75 °C. The freshly prepared coagulant solution was heated to 75 °C and then added slowly in a thin continuous stream with continuous gentle agitation till a clear whey separated out. Stirring was then stopped and the curd was allowed to remain in whey for about 5 minutes. It was then drained through a hang with muslin cloth (10 min) and stored for future use.

**2.4 Preparation of Herbal Sandesh:** Fresh chhana and herbs (table 1) was kneaded thoroughly to make an uniform dough. Fine powdered cane sugar (300 g) was added to the dough' and was kneaded again. The dough was then heated (75 °C) in an iron pan with continuous stirring. Heating was continued until the mixture acquired desired consistency with slightly cooked flavour. During the final stages of heating, the mixture developed slight cooked flavour and the sticking tendency to the pan disappeared. The cooking was completed in 15-20 min. The products were then transferred to a shallow pan, cooled and sliced into desired shapes. Thus, final product obtained and packed in plastic box for storage at room temperature ( $25 \pm 5$  °C).

**Table 1:** Ingredients Used in the Preparation of Herbal Sandesh for 1 Kg.

S. No.	Treatments	Chhana		Ashwagandha Root Extract		Tulsi Leave Extract		Total (gm)
		%	(gm)	%	(gm)	%	(gm)	
1	A <sub>0</sub> B <sub>0</sub>	100	1000	0	0	0	0	1000
2	A <sub>0</sub> B <sub>1</sub>	99	990	0	0	1	10	1000
3	A <sub>0</sub> B <sub>2</sub>	98	980	0	0	2	20	1000
4	A <sub>0</sub> B <sub>3</sub>	97	970	0	0	3	30	1000
5	A <sub>1</sub> B <sub>0</sub>	99	990	1	10	0	0	1000
6	A <sub>1</sub> B <sub>1</sub>	98	980	1	10	1	10	1000
7	A <sub>1</sub> B <sub>2</sub>	97	970	1	10	2	20	1000
8	A <sub>1</sub> B <sub>3</sub>	96	960	1	10	3	30	1000
9	A <sub>2</sub> B <sub>0</sub>	98	980	2	20	0	0	1000
10	A <sub>2</sub> B <sub>1</sub>	97	970	2	20	1	10	1000
11	A <sub>2</sub> B <sub>2</sub>	96	960	2	20	2	20	1000
12	A <sub>2</sub> B <sub>3</sub>	95	950	2	20	3	30	1000
13	A <sub>3</sub> B <sub>0</sub>	97	970	3	30	0	0	1000
14	A <sub>3</sub> B <sub>1</sub>	96	960	3	30	1	10	1000
15	A <sub>3</sub> B <sub>2</sub>	95	950	3	30	2	20	1000
16	A <sub>3</sub> B <sub>3</sub>	94	940	3	30	3	30	1000

**NOTE:** Sugar use For all Treatment: 300 gm (30.0% of total wt.)

## 2.5 Antioxidant activity

**DPPH assay:** Antioxidant activity of Herbal Sandesh was determined using stable radical, 1, 1-diphenyl-2-picrylhydrazyl (DPPH), as described by Brand-Williams *et al.* (1995)<sup>[4]</sup>. Antioxidant activity analyzed by Diphenylpicrylhydrazyl (DPPH) radical scavenging activity was determined method is based on the ability of the antioxidant to scavenge the DPPH cation radical. The hydrogen atoms or electrons donation ability of the corresponding extract was measured from the bleaching of purple colored MeOH solution of DPPH. This spectrophotometric assay uses stable radical 1, 1-Diphenyl-2-Picryl hydrazyl (DPPH) as a reagent. Briefly, 0.1 ml of sample extract or standard was added to 5 ml of DPPH reagent (0.00039 gm in 1 liter methanol) and vortexed vigorously. The reaction tubes were incubated in dark for 30 min, at room temperature and the discoloration of DPPH was measured against a reagent blank at 517 nm.

All values obtained are acquired from UV Spectrophotometer/colorimeter for assays:

$$\% \text{ Antioxidant activity} = \{( \text{absorbance at blank}) - (\text{absorbance at test}) / (\text{absorbance at blank})\} \times 100$$

**2.6 Free fatty acid (Oleic acid %):** Free fatty acid was conducted according to the method described by manual of methods of analysis of foods (milk and milk products) FSSAI, 2012.

**2.7 Statistical Analysis:** The data obtained were statistically analyzed for its validity by using factorial design and critical difference (C.D) technique<sup>[7]</sup>.

Number of treatments - 16

Number of replications - 5

Total number of samples - 80

## 3. Results and Discussions

### 3.1 Antioxidant Activity

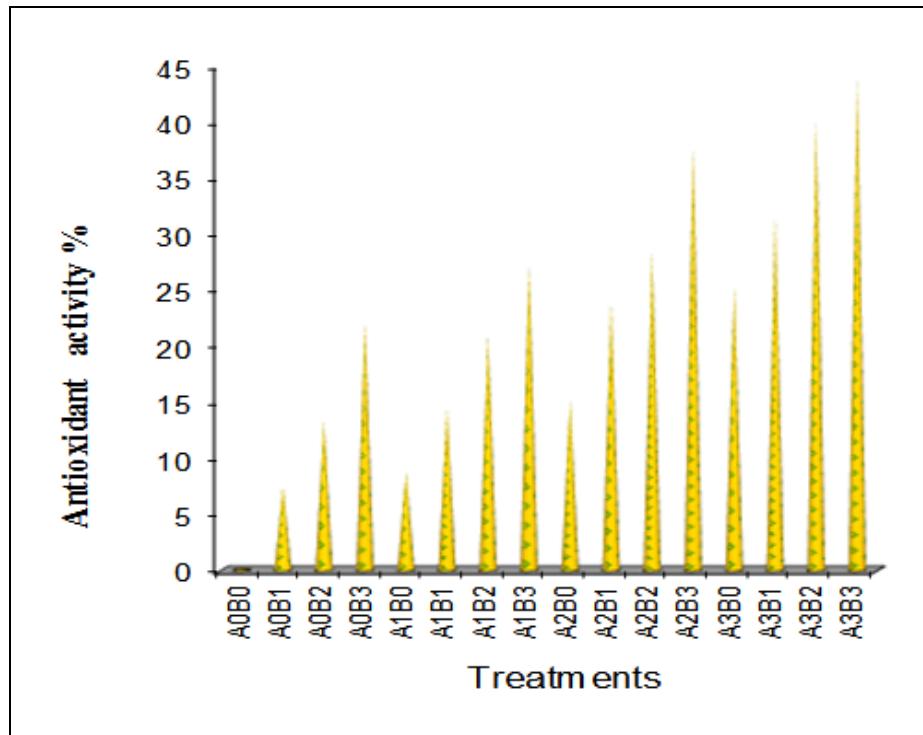
The assessment of antioxidant activity as affected by compositional parameters is indicated in Table 2, and Fig.1. The minimum was (0.00) obtained for the formulation that had Ashwagandha 0% and Tulsi 0% ( $A_0B_0$ ). The maximum percentage was (43.80) obtained for the formulation containing Ashwagandha 3%, Tulsi 3 % ( $A_3B_3$ ).

### 3.2 Free Fatty Acid (% Oleic acid)

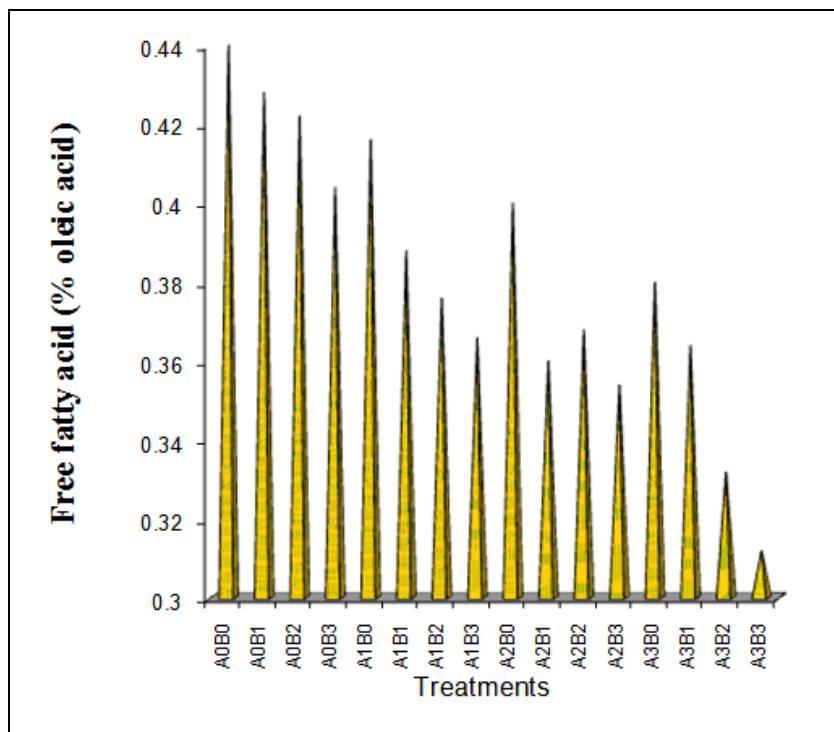
The assessment of free fatty acid percentage as affected by compositional parameters is indicated in Table 2, and Fig.2. The minimum percentage was (0.31) obtained for the formulation that had Ashwagandha 3% and Tulsi 3% ( $A_3B_3$ ). The maximum percentage was (0.44) obtained for the formulation containing Ashwagandha 0%, Tulsi 0 % ( $A_0B_0$ ).

**Table 2:** Antioxidant activity and free fatty acid of Herbal Sandesh (Mean Value)

S.No.	Treatments	Antioxidant Activity percentage	Free fatty acid (% Oleic acid)
1	$A_0B_0$	0	0.44
2	$A_0B_1$	7.48	0.43
3	$A_0B_2$	13.32	0.42
4	$A_0B_3$	22.15	0.40
5	$A_1B_0$	8.75	0.42
6	$A_1B_1$	14.52	0.39
7	$A_1B_2$	20.92	0.38
8	$A_1B_3$	27.25	0.37
9	$A_2B_0$	15.15	0.40
10	$A_2B_1$	23.91	0.36
11	$A_2B_2$	28.50	0.37
12	$A_2B_3$	37.82	0.35
13	$A_3B_0$	25.37	0.38
14	$A_3B_1$	31.60	0.36
15	$A_3B_2$	40.14	0.33
16	$A_3B_3$	43.80	0.31



**Fig 1:** Average of Antioxidant activity% (DPPH assay) in Herbal Sandesh

**Fig 2:** Average of Free fatty acid (% Oleic acid) in Herbal Sandesh

#### 4. Conclusion

The above research work provided a better understanding of desired Sensory evaluation imparted by the herbs on Herbal Sandesh. The Herbal Sandesh prepared by standard procedure incorporated with Ashwagandha and Tulsi extract. The antioxidant activity and free fatty acid (% Oleic acid) of Herbal Sandesh prepared by different levels of selected medicinal herbs can be determined accurately, conveniently, and rapidly using DPPH testing. The results shown above indicate that the herbal extracts gives high antioxidant activity, which was confirmed by method used for the antioxidant DPPH assay. It is concluded that the Herbal Sandesh containing 6 % Ashwagandha and Tulsi ( $A_3B_3$ ) 3% Ashwagandha and 3% Tulsi were higher as compared with other treatments in Antioxidant activity percentage and ( $A_3B_3$ ) were minimum free fatty acid (FFA) as compared with other treatments which also indicated good quality of Herbal Sandesh and having a great opening for marketing as value based product.

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