Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231-6345 (Online)
An Online International Journal Available at http://www.cibtech.org/jls.htm
2013 Vol. 3 (2) April-June, pp. 122-130/Manoharan and Ramasamy
Research Article

PHYSICO-CHEMICAL, MICROBIAL AND SENSORY ANALYSIS OF *ALOE VERA* (PULP) ICE CREAM WITH NATURAL COLOUR CURCUMIN IN DIFFERENT ARTIFICIAL SWEETENERS

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ABSTRACT
In India type -2 diabetic is increasing every year and make the people to go for alternative sugar. Ice cream is fat rich product enjoyed by all age groups. The demand for ice cream production is increasing 12 to 15 per cent every year in India. Curcumin is colouring pigment extracted from the turmeric having anti-microbial, anti-carcinogenic properties. The present investigation was undertaken to prepare herbal (*Aloe Vera*) ice cream with artificial sweeteners and curcumin natural colour to assess its acceptable level of the resultant product. *Aloe vera* pulp, artificial sweeteners and natural colour curcumin are added different level in ice cream and prepared ice cream is studied for its physico-chemical and microbial qualities.

Key Words: *Ice Cream – Aloe Vera – Artificial Sweeteners – Aspartame – Levulose – Sucralose – Food colour – Natural Colour – Curcumin – Physico-Chemical – Microbial Analysis.*

INTRODUCTION
Color becomes the most sensitive part of any commodity not only for its appeal but also it enhances consumer acceptability. In addition, the color of a food substance is important to indicate its freshness and safety that are also indices of good aesthetic and sensorial values. For natural color and additives, adherence to the norms of biosafety protocol, are limited. The demand for natural source of such compounds is increasing day by day because of awareness of positive health benefit out of natural compounds. The growing concern with health and the higher incidence of overweight, metabolic syndrome and diabetes have resulted in an increase in interest for foods with lipid and sugar reduction (Dabelea *et al.*, 2007). However, the development of new food products turns out to be increasingly challenging, as it has to fulfill the consumer's expectancy for products that are simultaneously relish and healthy (Cruz*et al.*, 2009).
The awareness of consumers for healthier food has led to the introduction in ice cream manufacture with fat replacers and high intensity sweeteners. Although, ice cream is a highly complex food matrix containing proteins, fat, sugars, air, minerals and countless interfaces between the different constituents (Frøst *et al.*, 2005). Thus, any modifications in the sugar composition and contents, besides using natural colours and flavours, can result in changes to the flavor and texture, which are key factors for the success of the ice cream on the market (Hatchwell, 1994).
High intensity sweeteners are successful if they match perfectly the quality of sucrose (Portmann and Kilcast, 1996). However, the replacement of sucrose by alternative sweeteners can be providing changes in the perception of bitter and sweet taste (Bolini-Cardello *et al.*, 1999). Alternative sweeteners should have a quick onset and a minimum persistence to mimick sucrose (Portmann and Kilcast, 1996). Therefore, the characteristics of interest related to the phsyscio-chemical, microbial and sensory quality of ice cream should be identified and properly studied by way of a sensory analysis (Minim *et al.*, 2006).
Aloe vera is one of the oldest known medicinal plants gifted by nature; *Aloe vera*, often called miracle plant is known by many names. There are over 200 types of *Aloe vera* and of these only 4 or 5 are commonly used in medicines. The most widely used variety of *Aloe vera* is *Barbadensismillar*. It is perennial, succulent plant with stiff fleshy leaves. *Aloe vera* is a clear thin gelatinous material that comes

from inside the *Aloe vera* leaves. *Aloe Vera* juice also improves blood circulation due to its ability to detoxify. It is also a natural healer, and hence any internal ulcers or lesions will be soothed and healing will be enhanced. *Aloe vera* leaves have vital ingredients such as vitamins, minerals, amino acids, polysaccharides, enzymes, plant steroids, saponins, lignin, anthraquinones, salicylic acid, which are necessary for the human body. *Aloe vera* works as anti-septic, antibacterial, antiviral, anti-carcinogenic and anti-inflammatory. It has been reported to cure eczema, diabetes, arthritis and is said to prevent infection. It also improves human immune system and digestive system.

Curcumin inhibits release of the pro-inflammatory cytokine TNF-alpha. Curcumin is a more effective anti-clotting agent than aspirin, without the ulcer-inducing stomach irritation caused by aspirin (Ben Best, 2006).

Turmeric is a bright yellow colourant made from the roots of the herb *Curcuma longa*. The pigments responsible for the colour are known as curcuminoids: curcumin and related compounds. Solubility of turmeric compound depends on the processing medium. Turmeric oleoresin is water soluble; but oil extract can be added to fat based foods. At high pH, the extract turns orange. There is no usage restriction as long as the level conforms to Good Manufacturing Practices (GMP) (Pritam *et al*., 2008).

The non-plant based adulterants in turmeric powder include artificial colours such as Metanil Yellow, Orange II and lead chromate which are detected by colourimetric, chromatographic or spectrophotometric techniques (Tripathi, *et al., 2004*)

In India, Rule 26 of The Prevention of Food Adulteration Rules (PFAR) permits 11 colours for food use: Lactoflavin, Caramel, Annato, Saffron, Curcumin etc., also approved by EU and FDA (Pritam *et. al.2008*).

In Asian countries, whole dry or fresh turmeric, ground or turmeric powder with other spices is used for making vegetable and meat preparations and soups (Sasikumar, 2001). Turmeric powder mixed with sesame, coconut or groundnut oil is used for making mango, lime, gooseberry, garlic and other pickles (Govindarajan, 1980).

Hence, producing an ice cream with reduced sugar and medicinal herbs (*Aloe vera* and curcumin) is deemed to fill a gap in the market and fulfill consumer demand. Hence, to study the physico-chemical and microbial properties of the herbal ice cream with artificial sweeteners.

MATERIALS AND METHODS

The present study was conducted at the modern dairy plant, Institute of Food and Dairy Technology, Koduvalli, Alamathi (post), Chennai. The raw materials used for the preparation of ice cream are as follows: Buffalo milk (5.0 per cent fat and 9.5 per cent MSNF) purchased from the nearby village; Butter (80 per cent fat) purchased from the Tamil Nadu Co-operative Milk Producers Federation Ltd., Aavin and was used to standardize the fat content of the ice cream. Skimmed milk powder (95 per cent MSNF) obtained from Tamil Nadu Co-operative Milk Producers Federation Ltd. Aavin was used to standardize the milk solids not fat (MSNF) content of ice cream. High quality stabilizers (gelatin) and emulsifiers (Glycein-mono-strate) were used for this research. Curcumin purchased from the M/s Cifal Herbal Pvt. Ltd. Gogineripuram, Gudur, 524 103 was used for colouring the butterscotch flavour ice cream. Butter scotch flavor was purchased from the Chemical Engineering Corporation Pvt. Ltd., Ponpadi, R.S and PO – 631 213, Tamil Nadu was used flavouring ice cream. High quality cane sugar (sucrose) was used.

Ice cream mix was prepared with 10 percent fat, 36 percent total solids, 0.5 per cent stabilizer and emulsifier in the ice cream, as per ISI (IS: 2802, 1964) specification (Sukumar De, 2008). The Artificial sweeteners like Levulose, Sucralose and Aspartame (along with dry mix) were added at the rate of 50 per cent. In each treatment, mix ingredients were homogenized as described by Arbuckle, (1986) and then heated to 80°C for 30 sec as suggested by Rothwell, (1976). Mixes were cooled to 5°C and aged overnight at the same temperature. The natural curcumin powder as sources of natural colour (0.5 per cent)and *Aloe vera* pulp were added at the rate of 15 per cent, 20 per cent and 25 per cent respectively before freezing. The different treatments carried out are shown in the following table. The freezing was

done in a batch freezer. The ice cream was filled in 50 ml paper cups, covered with lid and stored at –29°C.

The sensory characteristics of the ice cream samples were assessed using the ADSA IC score card. The sensory panel belongs to staffs and students of Institute of Food and Dairy Technology, Koduvalli, Chennai.

Items	**Perfect score**	**Score obtained**
Flavor	45	
Body and Texture	30	
Color	5	
Melting quality	5	
Bacterial count	15	
Total score	100	

ADSA IC Score card

The titrable acidity and pH of the ice cream were determined as per the procedure described in IS: 2802-1964.The standard plate count and coliform counts of the ice cream samples were determined as per the procedure described in IS: 2802-1964. The data collected were analyzed by analysis of variance (one way ANOVA) as described by Snedecor and Cochran (1989). And Duncan's multiple range tests were used as post hoc technique to study the significant difference among the means.

Milk
↓
Preheating 55-60°C
↓
Skim Milk Powder, Butter, Emulsifier, Stabilizer and Sugar (50%Cane sugar + 50% Artificial sweeteners),
↓
Preheating at 55-60°C
↓
Homogenization (2500 and 500 psi 1st and 2nd stage)
↓
Pasteurization 80°C for 30 seconds
↓
Cooling (4-5 °C and aging overnight)
↓
Mixing *Aloe vera* pulp, color and flavor and freezing
↓
Packing in ice cream cups
↓
Storage of ice cream (-29°C)

Figure 1: Process flow chart for the preparation of ice cream samples

Table 1: Sensory evaluation scores (Mean± SE)*for the ice cream prepared with different levels of *Aloe vera* pulp

Parameters	Control	*Aloe vera* pulp								
		5%	10%	15%	20%	25%	30%	35%	40%	45%
Flavour	41.32±0.314	42.12±0.431	41.85±0.409	41.26±0.428	39.50±0.212	38.02±0.302	38.61±0.144	38.76±0.150	38.56±0.176	38.21±0.209[a]
Color	4.92±0.027	4.80±0.056	4.79±0.056	4.80±0.054	4.81±0.058	4.91±0.042	4.91±0.043	4.78±0.059	4.80±0.049	4.72±0.056
Body & texture	28.41±0.154	27.35±0.216	27.52±0.184	27.64±0.176	28.80±0.117	27.71±0175	27.71±0.180	26.97±0.188	26.50±0.203	26.73±0.227
Melting quality	4.82±0.043	4.38±0.062	4.40±0.059	4.42±0.059	4.82±0.043	4.40±0.063	4.40±0.062	4.39±0.045	4.39±0.042	4.27±0.054
Microbial	15.00±0.000[a]	15.00±0.000[a]	15.00±0.000[a]	15.00±0.000[a]	15.00±0.000[a]	15.00±0.000[a]	15.00±0.000[a]	15.00±0.000[a]	15.00±0.000[a]	15.00±0.000[a]
Over all score	94.47±0.387	93.65±0.484	93.56±0.452	93.13±0.454	92.93±0.225	90.05±0.378	90.63±0.270	89.89±0.252	89.24±0.286	88.94±0.338

Means bearing different superscript in a row differ significantly (P< 0.01)
** Average of 8 trails*

Table 2: Sensory analysis score (Mean± SE)* for ice cream with curcumin as natural color for butterscotch flavour

Parameters	Control	Curcumin		
		0.3%	0.5%	0.7%
Flavour	38.70±0.146[c]	38.52±0.242[b]	39.38±0.172[c]	37.58±0.212[a]
Color	4.92±0.032[c]	4.23±0.052[a]	4.97±0.019[c]	4.41±0.056[b]
Body& Texture	28.18±0.123[b]	27.29±0.194[a]	28.18±0.155[b]	27.31±0.207a
Melting quality	4.80±0.046[c]	4.37±0.062[a]	4.18±0.046[c]	4.61±0.063[b]
Microbial	15.00±0.000[a]	15.00±0.000[a]	15.00±0.000[a]	15.00±0.000[a]
Overall score	92.22±0.203[b]	89.41±0.327[a]	92.34±0.240[b]	88.91±0.314[a]

Means bearing different superscript in a row differ significantly (P< 0.01)
** Average of 8 trails*

Table 3: Sensory analysis score (Mean± SE)*for ice cream with sucralose as artificial sweetener

Parameters	Control	Sucralose		
		50%	75%	100%
Flavour	43.24±0.206c	43.07±0.189c	38.52±0.242b	37.77±0.193a
Color	4.80±0.021b	4.92±0.027b	4.87±0.032b	4.47±0.068a
Body & texture	29.29±0.137b	29.31±0.101b	27.31±0.207a	26.87±0.194a
Melting quality	4.96±0.021c	4.91±0.029c	4.61±0.063b	4.38±0.064a
Microbial	15.00±0.00 [a]	15.00±0.00[a]	15.00±0.00 [a]	15.00±0.00 [a]
Overall score	97.44±0.256[c]	97.21±0.225[c]	90.37±0.328[b]	88.48±0.272[a]

Means bearing different superscript in a row differ significantly (P< 0.01)
** Average of 8 trails*

Table 4: Sensory analysis score (Mean± SE)*for ice cream with levulose as artificial sweetener

Parameters	Control	Levulose		
		50%	75%	100%
Flavour	42.96±0.250[c]	42.65±0.258[c]	38.97±0.101[b]	37.63±0.212[a]
Color	4.91±0.029 [b]	4.88±0.032 [b]	4.44±0.068 [a]	4.37±0.066 [a]
Body & texture	29.44±0.154[b]	29.35±0.132[b]	26.53±0.104[a]	26.56±0127 [a]
Melting quality	4.94±0.024[c]	4.87±0.037[c]	4.12±0.032 [b]	4.02±0.039[a]
Microbial	15.00±0.00 [a]	15.00±0.00 [a]	15.00±0.00 [a]	15.00±0.00 [a]
Overall score	97.26±0.275[c]	96.75±0.299[c]	89.07±0.144[b]	87.57±0.283[a]

Means bearing different superscript in a row differ significantly (P< 0.01)
** Average of 8 trails*

Table 5: Sensory analysis score (Mean± SE)*for ice cream with aspartame as artificial sweetener

Parameters	Control	Aspartame		
		50%	75%	100%
Flavour	43.61±0.223 [c]	43.37±0.261[c]	39.79±0.615[b]	38.14±0.242[a]
Color	4.78±0.067 [c]	4.66±0.071[bc]	4.51±0.065 [b]	4.21±0.065[a]
Body & texture	28.98±0.184[b]	28.89±0.136[b]	28.92±0.119[b]	25.28±0.123[a]
Melting quality	4.79±0.063 [c]	4.64±0.070[c]	4.27±0.070[b]	3.59±0.069[a]
Microbial	15.00±0.00 [a]	15.00±0.00 [a]	15.00±0.00 [a]	15.00±0.00 [a]
Overall score	97.16±0.300[c]	96.56±0.316[c]	92.49±0.638[b]	86.22±0.298[a]

Means bearing different superscript in a row differ significantly (P< 0.01)
** Average of 8 trails*

Table 6: Physico-chemical, microbial and sensory analysis of the *Aloe vera* (pulp) ice cream (Mean ± SE)* at various concentrations with natural color (curcumin) and natural identical butterscotch flavour in different artificial sweeteners

Parameters	Control	Aspartame			Levulose			Sucralose		
		15% Aloe	20% Aloe	25% Aloe	15% Aloe	20% Aloe	25% Aloe	15% Aloe	20% Aloe	25% Aloe
pH	6.54 ±0.030^{cd}	6.39±0.025^{bc}	6.41±0.007^{bcd}	5.96±0.102^{a}	6.57±0.041^{d}	6.52±0.034^{cd}	6.35±0.080^{b}	6.56±0.003^{cd}	6.52±0.023^{cd}	6.36±0.077
Titrable acidity	0.23 ±0.004^{ab}	0.19±0.003^{a}	0.21±0.016^{ab}	0.32±0.028^{c}	0.22±0.013^{ab}	0.24±0.002^{b}	0.29±0.014^{c}	0.24±0.003^{b}	0.25±0.009^{b}	0.29±0.018^{c}
SPC (log 10 cfu/ml)	2.76 ±0.466^{b}	2.76±0.327^{b}	2.29±0.239^{ab}	1.41±0.364^{a}	2.71±0.294^{b}	1.47±0.315^{a}	1.35±0.402^{a}	2.24±0.315^{ab}	1.35±0.331^{a}	1.29±0.381^{a}
Overall organoleptic quality	95.51±0.468^{d}	89.84±0.565^{b}	94.57±0.571^{cd}	88.13±0.502^{a}	89.51±0.569^{ab}	93.66±0.512^{c}	88.66±0.577^{ab}	88.94±0.397^{ab}	95.15±0.522^{cd}	87.93±0.443^{a}

Means bearing different superscript in a row differ significantly (P< 0.01)
** Average of 8 trails*

RESULTS AND DISCUSSION

The average sensory scores presented in table 1 reveals that the incorporation of *Aloe vera* pulp in the ice cream significantly diminished the overall sensory scores compared to the control ice cream. The average scores for flavour, colour, body and texture and melting quality were significantly lower than the control samples. The aim of the study was to include maximum amount of *Aloe vera* pulp in the ice cream since it contains very low in total solids but rich in all micronutrients essential for mankind, but is bitter in taste. Hence inclusion of *Aloe vera* pulp in ice cream reduces the sensory characters viz., colour, flavour, which can be overcome by using proper flavour and colour. The sensory evaluation scores revealed that acceptability was highest for the ice cream mix incorporated with 20 per cent *Aloe vera* pulp. Hence, its next lower and higher level of incorporation viz., 15 per cent and 25 per cent along with 20 per cent *Aloe vera* pulp were taken up for further studies.

Curcumin is a potent anti-oxidant, anti-carcinogenic and anti-inflammatory agent and is a more effective anti-clotting agent than aspirin, without the ulcer-inducing stomach irritation caused by aspirin (Ben Best, 2006). In India, Rule 26 of The Prevention of Food Adulteration Rules (PFAR) permits the use of curcumin as food colouring agent (Pritam *et al.*, 2008). Preliminary screening tests conducted by visual perception revealed that 0.5 percent curcumin was the optimum level of incorporation in butterscotch flavoured ice creams. Hence, its immediate lower and higher level of incorporation ie. 0.3 percent and 0.7 percent along with 0.5 percent were taken up for sensory evaluation. Table 2 shows the average sensory analysis scores for the curcumin powder as natural colour for the butterscotch flavoured ice cream. The overall average score for the control samples was 92.22 and the inclusion of curcumin powder at 0.3, 0.5 and 0.7 percent obtained the scores of 89.41, 92.34 and 88.91, respectively. The inclusion of curcumin powder at both 0.3 and o, 7 % levels in the butterscotch flavoured ice cream significantly diminished the sensory while at the inclusion level of 0.5 percent curcumin enhanced the sensory scores of butterscotch

flavoured ice cream, though not significantly in comparison to that of control samples. Pritam *et al*. (2008) suggested that there is no usage restriction as long as the level conforms to Good Manufacturing Practices (GMP), it was concluded that 0.5 percent curcumin was the optimum level of inclusion in butter scotch flavour ice cream.

In this study, sucralose was substituted for cane sugar in the ice cream samples at three different levels viz., 100, 75 and 50 per cent and the average sensory scores for different characters and the overall average scores are presented in table 3. It may be noticed that the inclusion level of 50 per cent had the maximum overall score and was statistically similar to that of control samples for all the sensory characters. It was also found that sucralose when substituted for cane sugar at 75 and 100 percent level adversely affected the body and texture and melting quality apart from flavour which might be due to the reduction in the bulk volume of the ice cream. Sucralose has no calorific value, is not metabolized by the body, can be used in cooking and baking, and an average daily intake (ADI) of 15 mg per kg of bodyweight is permissible in human beings. (Marie Spano, 2002). Hence the optimum level of substitution (50 percent) as found in the present study is well within the ADI.

Table 4 shows the average sensory analysis scores of ice creams incorporated with levulose in place of cane sugar at different levels in the ice cream. The overall average sensory scores for 100, 75 and 50 per cent inclusion levels were 87.57, 89.07 and 96.75, respectively; while the corresponding value for control samples were 97.26. All the sensory characters were significantly altered by the level of inclusion of levulose in the ice cream samples. The inclusion of levulose at the level of 50 per cent was statistically different from the remaining two levels of inclusion and was not significantly different from the control samples. The results of the study showed that levulose can be added with the ice cream mix at the rate of 50 per cent.

Aspartame was substituted for cane sugar in ice cream in three levels viz., 100, 75 and 50 per cent. The overall average values scored by three levels of inclusion namely 100, 75 and 50 per cent were 86.22, 92.49 and 96.56, respectively. The control samples had the maximum overall average score of 97.16 and all the sensory characters were not statistically different from the samples prepared with 50 percent aspartame as artificial sweetener (Table 5). However the other two levels of inclusion viz., 100 and 75 percent significantly altered the sensory scores of the all the characters and hence the 50 per cent level of inclusion of aspartame in the ice cream was considered as optimum. Aspartame is a dipeptide (L-aspartyl-Lphenylalanyl-methyl ester) with a sweetening power of 180 to 200 times that of sucrose (Mazur RH, 1969). The Joint Expert Committee on Food Additives (JECFA) estimated the level causing no effect in the rat to be 4 g/kg body wt /day and proposed an ADI (safety factor 100) of 40 mg/kg body wt/day for humans (JECFA, 1980). Hence, it was found that aspartame can be substituted for cane sugar at 50 percent level without affecting the sensory characters of ice cream which was also well within the ADI.

The physico-chemical, microbial and sensory analysis results of ice cream samples added with curcumin as natural colour and butterscotch as natural identical flavour are presented in table 6. *Aloe vera* was included at three different levels viz. 15, 20 and 25 percent with three artificial sweeteners viz., aspartame, levulose and sucralose. The mean pH value of control samples was 6.54 and for the samples with artificial sweeteners viz., aspartame, levulose and sucralose in 15, 20 and 25 percent inclusion of *Aloe vera* were 6.39, 6.41, 5.96, 6.57, 6.52, 6.35; 6.56, 6.52 and 6.36 respectively. The corresponding values for titrable acidity were 0.23; 0.19, 0.21; 0.32; 0.22, 0.24, 0.29; 0.24, 0.25 and 0.29, respectively. The mean standard plate count of the control samples was 2.76. The samples with 15, 20 and 25 percent of *Aloe vera* and aspartame, levulose and sucralose were 2.76, 2.29, 1.41; 2.71, 1.47, 1.35; 2.24, 1.35 and 1.29, respectively.

The results of the present study revealed that the increased level of inclusion of *Aloe vera* in the ice cream reduced the pH value and standard plate count and simultaneously increased the titrable acidity. *Aloe vera* contains uronic acid, salicylic acid and phenolic compounds which probably caused the increase in the acidity and thereby a decrease in the pH of the ice cream. The ice cream was prepared in the department dairy plant under strict hygienic precautions and hence coliforms were not found in the sample. This may

be also due to the presence of Aloe vera which was having anti-microbial properties. In addition to the above *Aloe vera* also contains many anti-bacterial, anti-viral and anti-fungal agents namely saphonins, fatty acids etc. which might have caused in the reduction of standard plate count (Klien and Penneys, 1988; Marshall, 1990; Ahmad *et al*., 1993).

Conclusion

The results of the present study of one way analysis of variance revealed that the incorporation of *Aloe vera* pulp in the ice cream significantly diminished the sensory scores. The average scores of flavour, colour, body and texture and melting quality were significantly (P<0.01) lower than the control samples. The sensory evaluation scores revealed that acceptability was highest for the ice cream mix incorporated with 20 per cent *Aloe vera* pulp. Hence, its next lower and higher level of incorporation viz., 15 per cent and 25 per cent along with 20 per cent *Aloe vera* pulp were taken up for further studies. The addition of various levels of natural curcumin colour in the ice cream significantly (P<0.01) altered the sensory qualities and the optimum inclusion levels was found to be 0.5 per cent. Three artificial sweeteners namely aspartame, levulose and sucralose were also added to substitute cane sugar at 100, 75 and 50 per cent. Significant difference (P<0.01) was noticed between the various levels of substitution of artificial sweeteners and it was found that the artificial sweeteners can be substituted for cane sugar at the rate of 50 per cent without much altering sensory properties of the ice cream. Three different concentration of *Aloe vera* pulp incorporated in the ice cream sucralose as artificial sweetener at 20 per cent *Aloe vera* pulp scored 94.70 followed by the aspartame and levulose respectively.

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