Annals of Agri-Bio Research 19 (4) : 780-784, 2014

Development and Storage Studies of Aloe Vera Ice-cream

KULVEER SINGH AHLAWAT*, B. S. KHATKAR**, NEELAM GULIA AND SUSHIL[1]

Department of Food Technology, Guru Jambheshwar University of Science & Technology, Hisar-125 001 (Haryana), India
**(e-mail : kulveersushil@yahoo.co.in; **bhup2009@hotmail.com)*

ABSTRACT

Five formulae of aloe vera ice-cream were developed in this study. Aloe vera was incorporated in the form of aloe vera cubes, aloe vera cubes coated with sugar, aloe vera gel, aloe vera gel concentrate and aloe vera gel powder. Other ingredients like sweetening, colouring, and flavouring agents were also added. Sensory attributes were evaluated using 9-point Hedonic Scale method. Aloe vera ice-cream made by using aloe vera gel obtained the overall acceptability score of 7.52 ("like moderately" to "like very much") which was much higher than that of other four formulae. The overall acceptability score was 6.49 for aloe vera gel concentrate containing formula, 6.35 for aloe vera gel powder containing formula, 6.20 for aloe vera cubes coated with sugar ("like slightly" to "like moderately") and 5.40 for aloe vera cubes containing formula ("neither like nor dislike" to "like slightly"). Storage study revealed that aloe vera gel containing ice-cream was acceptable after three months storage period at -18 and -25°C.

Key words : Aloe vera gel, ice-cream, acemannan, therapeutic value, aloe vera gel cubes

INTRODUCTION

Aloe vera has been used therapeutically since Roman times and perhaps long before (Morton, 1961; Crosswhite and Crosswhite, 1984). Aloe vera contains two major liquid sources, yellow latex (exudate) and the clear gel (mucilage). Yellow latex is mainly composed of aloin, aloe-emodin and phenols. The mucilaginous jelly from the parenchyma cells of the plant is the aloe vera gel (Lawless and Allan, 2000). Besides the use for treatment of thermal and radiation burns locally (Collin and Collin, 1935; Wright, 1936), aloe vera gel exhibits anti-inflammatory and antidiabetic activity (Brudner and Baranova, 1972; Shinpo *et al.*, 1978). Two controlled clinical studies had shown that aloe vera, either alone or in combination with the oral hypoglycemic drug, glibenclamide effectively lowered blood sugar in people with diabetes mellitus (Bunyapraphatsara *et al.*, 1996). Aloe vera gel was found effective against pulmonary carcinogenesis, stomach and colon cancer (Sakai, 1989). Antibacterial, antifungal and anti-viral activities have also been demonstrated by the gel (Klien and Penneys, 1988; Marshall, 1990; Ahmad *et al.*, 1993). Acemannan, a polysaccharide fraction of aloe vera gel, was used to treat AIDS patients. A 71% reduction in symptoms was recorded, perhaps due to stimulation of the immune system (McDaniel *et al.*, 1987). The gel polysaccharides exhibited immunomodulatory properties and the acemannan has reached proprietary status (McAnalley, 1988; Agarwala, 1997). Aloe gel is offered commercially for oral consumption and many claims are made for benefits in various internal inflammatory conditions. A review on aloe vera included the effects on gastrointestinal function and ulcers (Reynolds and Dweck, 1999). A series of trials on patients indicated a tonic effect on the intestinal tract with a reduced transit time. Also the intestinal microbial flora appeared to be benefited, with a reduction in the presence of yeasts and a reduction in pH. Bowel putrefaction was reduced and protein digestion/absorption improved (Bland, 1985). An early clinical trial showed that oral administration of aloe gel was effective in the treatment of peptic ulcers (Blitz *et al.*, 1963). In a randomized, double-blind, placebo-controlled trial, aloe vera gel administered orally for active ulcerative colitis, at 100 ml, twice daily for four weeks, produced a clinical response more often than placebo and reduced the histological disease activity as well (Langmead *et al.*, 2004). According to pharmacological studies, a lectin fraction (glycoprotein), aloctin A was effective against gastric lesions in rats (Saito, 1993). Health benefits of aloe vera also include increasing high density lipoproteins (HDL) level and reducing low density lipoproteins (LDL) level (Ahlawat and Khatkar, 2011). The list of therapeutic benefits of aloe vera gel is very long. This encouraged the people around globe to accept aloe vera as a functional food component and its incorporation in food products was taken up on a wider

[1]Department of Entomology, CCS Haryana Agricultural University Hisar-125 004 (Haryana), India.

scale. It was observed that major limitation of aloe vera gel and its products was their bland taste. The aloe vera can be made in a more palatable form by incorporating it in frozen foods like ice-cream. Later is a frozen dairy food made by freezing a pasteurized mix with agitation to incorporate air and ensure uniformity of consistency. The mix is composed of a combination of milk, sugar, dextrose, corn syrup, water or other optional ingredients, with or without eggs or egg products, with or without flavourings, and with or without added stabilizers or emulsifiers and other ingredients.

Since aloe vera ice-cream is a frozen product, the active ingredients of aloe gel would remain intact and functional. Furthermore, frozen products from aloe gel would be another alternatives for consumers, apart from the commercially available aloe drinks. The purpose of this study was, therefore, to develop an aloe frozen product in the form of soft ice cream using aloe gel in different forms. Sensory evaluation of the developed products was carried out. The best rated product in term of sensory quality was studied for shelf life stored at -18°C and -25°C. Proximate composition of the standard product was also determined.

MATERIALS AND METHODS

The healthy disease-free aloe vera leaves from 4-year mature plants (optimum maturity) were harvested from aloe vera plantation raised at local herbal park. Four-year old plant's leaves were selected to recover highest gel yield (Ahlawat *et al.*, 2013a). The leaves were washed, trimmed, drained and filleted as per method of Ahlawat *et al.* (2013b). The aloe vera leaf pulp so obtained was then prepared into five forms as follows :

Aloe vera cubes : The washed leaf pulp was cut into $0.5 \times 0.5 \times 0.5$ cm^3 cubes. The cubes were then stored at 4°C until further use.

Sugar coated aloe vera cubes : The washed leaf pulp was prepared into cubes described as above. The aloe vera cubes were mixed with ground sugar in the proportion of 1:1 by weight. The prepared cubes were then stored at 4°C for at least 24 h.

Aloe vera gel : The pulp was blended in a blender. The slurry was then filtered through a muslin cloth. The gel so obtained was pasteurized at 85°C for 30 min and then stored at 4°C until further use.

Aloe vera concentrate : It was prepared by concentrating the gel using a rotary evaporator. The concentrate was stored at 4°C till further use.

Aloe vera gel powder : It was prepared by freeze drying of aloe vera gel. The powder was stored in an airtight container at 4°C till further use.

Five different ice-creams were prepared based on addition of aloe vera in five different forms. The aloe vera gel, aloe vera cubes and sugar coated aloe vera cubes were added at a level of 25% (w/w). While the aloe vera gel concentrate was added at a level of 5% (w/w) and aloe vera gel powder at level of 1% (w/w) in the ice-cream base formula. The above levels were decided in order to meet the recommended daily intake of aloe vera in different forms. Other ingredients like full cream milk, cream, skimmed milk powder, butter, sucrose, lemon flavour and green colour were procured from local market. Stabilizer carboxy methyl cellulose (CMC) was received from department's store. The base formula used for preparation of ice-cream was as per an industrial recipe which has been elaborated in Table 1. For preparation of ice-cream containing aloe vera gel the liquid ingredients (water, cream, whole milk, etc.), except aloe vera gel, were mixed and heated to 45°C. The dry ingredients (skimmed milk powder, sucrose, stabilizer, etc.) were separately mixed in another container. The mixed dry ingredients were gradually added to the liquid mixture with constant stirring. Butter was cut into small pieces and added to the mix and allowed to completely melt before the pasteurization temperature reached. The mixture was then heated and pasteurized at 68°C for 30 min. It was then homogenized and later cooled to 4°C. The cooled mixture was aged at 4°C for 2 h. Aloe gel was then added and mixed. Colouring and flavouring agents were also added. The mixture was then transferred into a home scale ice-cream maker and freezing process was carried out until soft textured ice cream was obtained.

Table 1. Base formula used for preparation of ice-cream

S. No.	Ingredients	Amount (% w/w)
1.	Milk (Standardized)	49.64
2.	Cream	4.67
3.	Butter (Without salt)	3.75
4.	Skimmed milk powder	3.75
5.	Sucrose	12.75
6.	Stabilizer (Carboxy methyl cellulose)	0.23
7.	Lemon flavour	0.14
8.	Green colour	0.07
9.	Water	q. s.

The ice-creams with aloe vera cubes, aloe vera cubes coated with sugar, aloe vera gel concentrate and aloe vera gel powder were prepared by similar method. The best rated sample was reproduced in sufficient quantities, packed in 50 ml quantity in plastic ice-cream cups and kept for shelf life studies. For storage studies, samples were divided into two lots one stored at -8°C and other at -25°C for a period of 90 days. Observations for changes in sensory attributes were recorded after a period of every 30 days.

The fresh aloe vera gel was analysed for its physico-chemical characteristics. Specific gravity and refractive index were measured at 20°C using pycnometer and an automatic refractometer, respectively. Viscosity was measured at 20°C with Brookfield viscometer using spindle number 2 at 60 rpm. Digital pH meter was used for pH determination. Titratable acidity (TA), total sugars (TS), total solids, moisture content, reducing sugars, ash content, lipid content, protein content and fiber content were determined as per methods described by Ranganna (1986). The total soluble solids (TSS) were measured by Erma hand refractrometer of 0-32°B range. The bioactive principal, acemannan content was estimated by method of McAnalley (1988). The best rated ice-cream sample was also evaluated for proximate composition as per standard methods.

The sensory attributes of developed aloe vera ice-cream were evaluated by a sensory panel comprising 10 semi-trained panelists selected from the students, teachers and employees of the Department of Food Technology, GJUS & T, Hisar. The panelists (10) were asked to assign appropriate score to each formula of ice cream based on 1 to 9-Point Hedonic Scale (Amerine *et al.*, 1965) for taste, flavour, body, texture and overall acceptability (Table 2). The ice-cream samples were served frozen. The scale was such that : 9=Like extremely, 8=Like very much, 7=Like moderately, 6=Like lightly, 5=Neither like nor dislike, 4=Dislike slightly, 3=Dislike moderately, 2=Dislike very much and 1=Dislike extremely.

Table 2. Sensory quality of different formulae of *Aloe vera* ice-cream

Ice-cream formulae	Taste	Flavour	Texture	Body	Overall acceptability
Aloe vera cubes	5.91	6.23	5.62	5.30	5.40
Sugar coated aloe vera cubes	6.32	6.30	5.73	5.94	6.20
Aloe vera gel	7.40	7.53	7.81	7.72	7.52
Aloe vera gel concentrate	6.51	6.22	7.63	7.32	6.49
Aloe vera gel powder	6.60	6.40	6.13	6.33	6.35

Data represented are means of 10 values.

RESULTS AND DISCUSSION

Perusal of data presented in Table 3 indicates that fresh aloe vera gel had high percentage of water and low values for TSS and total sugar. It was found acidic in nature. Different forms of aloe vera exhibited different attributes when incorporated in ice-cream.

Aloe vera cubes : The cubes of aloe leaf pulp were clear, soft, with thin green line in some of the cubes. When the cubes were mixed with ice-cream base ormula (Table 1) at 25% w/w, and subsequently rozen, undesirable ice flakes were formed on the outer rface of the cubes. Furthermore, the bland flavour the cubes contrasted very drastically with the sweet vour of the ice-cream base.

e vera cubes coated with sugar : After mixing h the ice-cream base, the cubes as well as the sugar tended to precipitate to the bottom of the ice-cream container after the freezing process. Similar to the cubes without sugar, the cubes with sugar gave bland flavour contrasting to the sweet flavour of ice-cream

Table 3. Physico-chemical characteristics of fresh aloe vera gel

Physico-chemical properties	Values
Specific gravity	1.0064±0.00
Refractive index	1.3348±0.00
Viscosity (cP)	52.70±0.29
pH	4.81±0.00
Titrable acidity as malic acid (%)	0.47±0.02
Total solids (%)	1.10±0.01
Total sugars (%)	0.27±0.01
Total soluble solids (°B)	1.00±0.00
Acemannan (%)	0.20±0.02

*Standard deviation for specific gravity and refractive index was almost zero.

Data represented are means of three values.

base. In an attempt to include aloe vera pulp as 0.5 × 0.5 × 0.5 cm^3 cubes either with or without coating in ground sugar, it was found that the aloe pulp cube's blandness was not well compatible with the ice-cream base. Furthermore, the sugar coated cubes exhibited ice crystal formation on the outer surface of the cubes which was undesirable.

Aloe vera gel : The aloe gel obtained was clear and viscous. After mixing with the ice-cream base formula, the clear gel blended very well into the ice-cream base. The texture of the finished ice cream was smooth. The flavours of the gel and the ice-cream base were well compatible.

Thus, aloe gel was the form that was found to be most suitable for incorporation in the ice-cream. The gel also blended well with the ice-cream base.

Aloe vera gel concentrate : The aloe vera gel concentrate was also blended well with ice-cream base giving smooth texture to the final product.

Aloe vera gel powder : Aloe vera gel powder showed clumps in the ice-cream with some spots of little bitterness.

Ice-cream formula containing aloe vera gel obtained the highest average score of 7.52 ("like moderately" to "like very much"). The average overall acceptability score was much higher than the other four formulae i. e. aloe vera cubes containing ice-cream, sugar coated aloe vera cubes containing ice-cream, aloe vera gel concentrate containing ice-cream and aloe vera gel powder containing ice-cream which obtained the average scores of 5.40 ("neither like nor dislike" to "like slightly"), 6.20, 6.49 and 6.35 ("like slightly" to "like moderately"), respectively.

Proximate composition (Table 4) of aloe vera gel ice-cream showed good nutritional value. On storage, sandiness was observed due to development of large ice crystals. The ice-cream was acceptable after the storage period of 90 days under both the temperature of storage (Table 5).

Table 4. Proximate composition of standard Aloe vera ice-cream

Constituent	Amount (%)
Moisture	73.74
Protein	3.55
Fat	6.18
Total sugar	14.71
Total ash	0.68
pH	6.20

Data represented are means of three values.

Table 5. Effect of storage intervals on sensory quality of aloe vera ice-cream stored at -18°C and at -25°C for a period of 90 days

Characteristics	Storage period (days)							
	0 day		30 days		60 days		90 days	
	-18°C	-25°C	-18°C	-25 °C	-18°C	-25 °C	-18°C	-25°C
Colour	7.35	7.40	7.10	7.30	6.95	7.10	6.90	7.00
Taste	7.40	7.50	7.20	7.35	6.55	6.70	6.30	6.59
Flavour	7.53	7.52	7.40	7.45	7.30	7.35	7.15	7.30
Appearance	7.62	7.65	7.58	7.63	7.40	7.50	7.35	7.47
Overall acceptability	7.52	7.50	7.33	7.47	7.13	7.33	6.57	7.14

Data represented are the means of 10 values.

CONCLUSION

Ice-cream was selected as potential vehicle for the incorporation of aloe vera in five different forms i. e. aloe vera gel, aloe vera cubes, sugar coated aloe vera cubes, aloe vera gel concentrate and aloe vera gel powder. Aloe vera ice-cream containing aloe vera gel obtained the highest average sensory score and should be the potential formula for further development at commercial scale. Aloe ice-cream might be a good alternative apart from the products in the form of aloe gel juice, drinks, or canned cubes in light syrup which are currently available in the market. Since all formulae of the aloe ice-cream consisted of sucrose as the sweetening agent, clinical uses should be emphasized for activities other than the antidiabetic activity. Special formulae for diabetic patients may be further developed with the replacement of sucrose by other intense sweeteners.

REFERENCES

Agarwala, O. P. (1997). Whole leaf aloe gel vs. standard aloe gel. *Drug and Cosmetics Industry.* pp. 8-22.

Ahlawat, K. S. and Khatkar, B. S. (2011). Processing, food applications and safety of aloe vera products : a review. *J. Food Sci. Technol.* **48** : 525-533.

Ahlawat, K. S., Khatkar, B. S., Sushil and Gulia, N. (2013b). Effect of processing and storage on sensory properties and acemannen content of aloe vera gel. *Annals of Agri-Bio Res.* **18** : 70-73.

Ahlawat, K. S., Sushil, Gulia, N. and Khatkar, B. S. (2013a). Effect of plant maturity on leaf growth, yield and physio-chemical properties of aloe vera gel. *Agro Food Ind. Hi-Tech.* **24** : 35-37.

Ahmad, S., Kalhoro, M. A., Kapadia, Z. and Badar, Y. (1993). Aloe, a biologically active and potential medicinal plant. *Hamdard Medicos* **36** : 15-108.

Amerine, M. A., Pangborn, R. M. and Roessler, E. B. (1965). *Laboratory Studies : Quantity-quality Evaluation.* Academic Press, New York. pp. 349-397.

Bland, J. (1985). Effect of orally consumed *Aloe vera* juice on gastrointestinal function in normal humans. *Preventive Medicine* **14** : 4-152.

Blitz, J., Smith, J. W. and Gerard, J. R. (1963). *Aloe vera* gel in peptic ulcer therapy : Preliminary report. *J. Am. Osteo. Assoc.* **62** : 5-731.

Brudner, I. K. and Baranova, L. L. (1972). Treatment of acne conglobata. *Zdravookhr Beloruss.* **18** : 3-62.

Bunyapraphatsara, N., Jirakulchaiwong, S., Thirawaraphan, S. and Manonukul, J. (1996). Antidiabetic activity of *Aloe vera* L. juice. II. Clinical trial in diabeties mellitus patients in combination with glibenclamide. *Phytomedicine* **3** : 8-245.

Collin, C. and Collin, C. (1935). Roentgen dermatitis treated with fresh whole leaf species of *Aloe vera. Am. J. Roentgen* **33** : 7-396.

Crosswhite, F. S. and Crosswhite, C. D. (1984). *Aloe vera*, plant symbolism and the threshing floor. *Desert Plants* **6** : 43-50.

Klein, A. D. and Penneys, N. S. (1988). *Aloe vera. J. Am. Acad. Dermtol.* **18** : 20-714.

Langmead. L., Feakins, R. M., Goldthorpe, S., Holt, H., Tsironi, E., De Silva, A., Jewell, D. P. and Rampton, D. S. (2004). Randomized, double-blind, placebo-controlled trial of oral *Aloe vera* gel for active ulcerative colitis. *Alimen. Pharmacol. Ther.* **19** : 47-739.

Lawless, J. and Allan, J. (2000). *Aloe vera*–natural wonder cure. Harper Collins Publishers, London.

Marshall, J. M. (1990). *Aloe vera* gel : What is the evidence? *Pharmaceutical J.* **2** : 360.

McAnalley, B. H. (1988). Process for preparation of aloe products, produced thereby and composition thereof. *US Patent* **4** : 735,935.

McDaniel, H. R., Perkins, S. and McAnalley, B. H. (1987). A clinical pilot study using carrisyn in the treatment of acquired immunodeficiency syndrome (AIDS). *Am. J. Clin. Pathol.* **88** : 534-537.

Morton, J. F. (1961.) Folk uses and commercial exploitation of *Aloe* leaf pulp. *Econ. Bot.* **15** : 9-311.

Ranganna, S. (1986). *Handbook of Analysis and Quality Control for Fruit and Vegetable Products.* Tata McGraw Hill, New Delhi.

Reynolds, T. and Dweck, A. C. (1999). *Aloe vera* leaf gel : a review update. *J. Ethnopharmacol.* **68** : 3-37.

Saito, H. (1993). Purification of active substances of *Aloe arborescens* Miller. and their biological and pharmacological activity. *Phytotherapy Res.* **7** : 9-14.

Sakai, R. (1989). Epidemiologic survey on lung cancer with respect to cigarette smoking and plany diet. *Jap. J. Can. Res.* **80** : 20-513.

Shinpo, M., Kameyama, S. and Tokita, F. (1978). Antiinflammatory topical compositions containing lidocaine and diphenylhydramine. *US Patent* **4** : 748, 022.

Wright, C. S. (1936). *Aloe vera* in the treatment of roentgen ulcers and telangicetasis. *Jama.* **106** : 4-1363.

Annals of Biology 30 (4) : 705-710, 2014

Development and Shelf Life Studies of Aloe Vera-Guava Jelly

KULVEER SINGH AHLAWAT*, B. S. KHATKAR**, SUSHIL[1] AND NEELAM GULIA

Department of Food Technology, Guru Jambheshwar University of Science & Technology, Hisar-125 001(Haryana), India

**(e-mail : kulveersushil@yahoo.co.in; **bhup2009@hotmail.com)*

ABSTRACT

In this study, efforts were made to prepare jelly by a combination of aloe vera gel with guava. Physico-chemical characteristics of aloe vera-guava pectin extracts (AGPE), aloe vera gel and aloe vera-guava jelly were determined. The first aloe vera-guava pectin extract was analyzed for moisture content (95.5%), ash (0.35%), acidity (0.91%), vitamin C (55.47 mg/100 g), total sugar (3.00%), TSS (3.5°Brix) and pH (3.46). The chemical composition of aloe vera-guava jelly prepared from composite of first and second aloe vera-guava pectin extract was moisture 21.53%, ash 0.28%, TSS 67%, total sugar 64%, acidity 0.78% and pH 3.20. Storage study of the jelly was conducted for three months for samples stored at ambient temperature (30±2°C) and under refrigerated temperature (4°C). It was observed that TSS, colour and flavour of jelly did not show any remarkable changes but pH and acidity showed small changes over the storage period.

Key words : Aloe vera-guava jelly, sensory quality, proximate composition, storage

INTRODUCTION

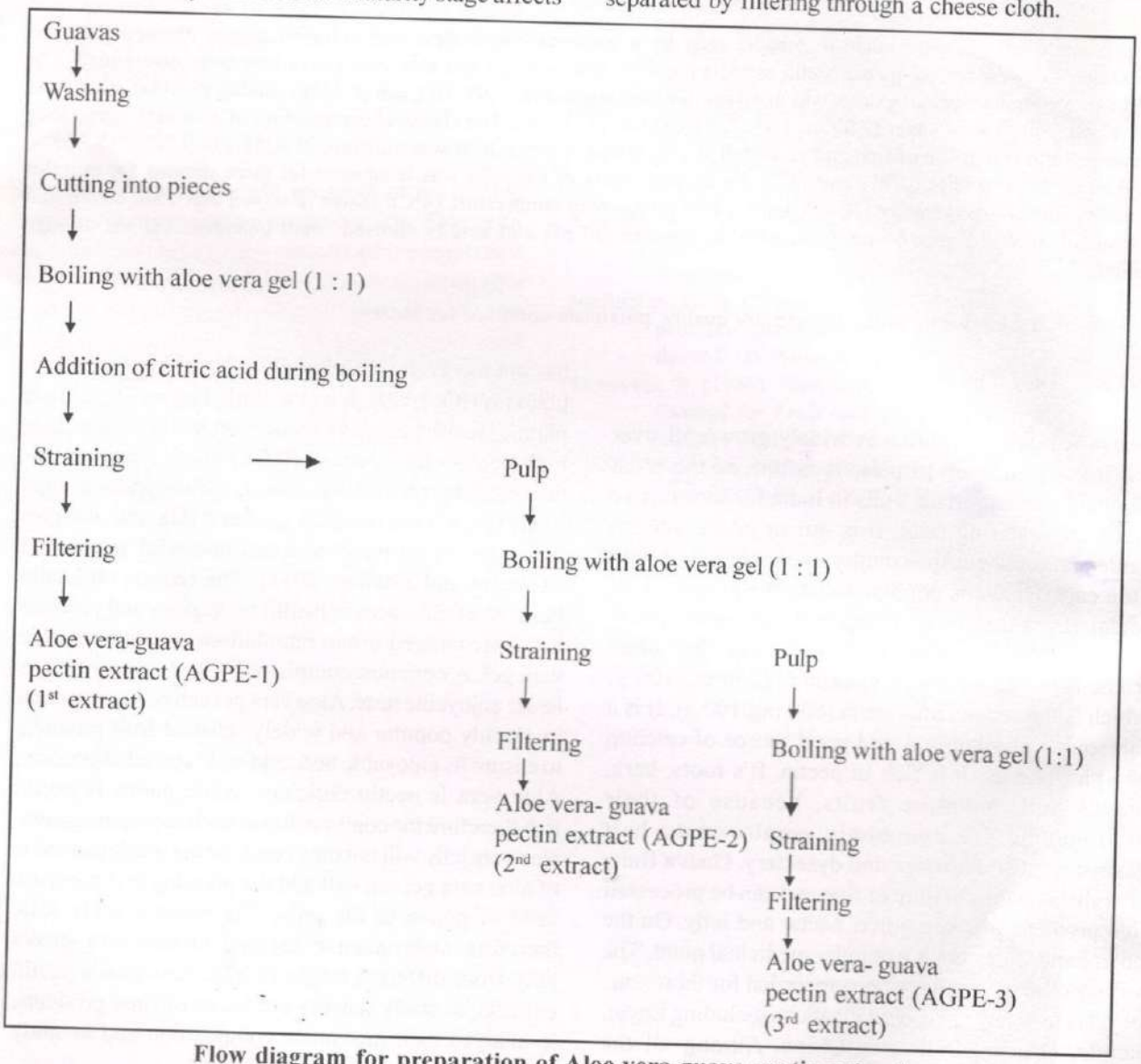
Guava (*Psidium guajava*) is widely grown all over the tropics and sub-tropics. It is one of the most common and important fruits in India for its nutritive value and pleasing taste. It is grown in the kitchen gardens throughout the country even without or with little care. Guava is popular among the people of all social strata due to its comparatively lower price, pleasing taste and good nutritional value than other fruits. It is rich source of vitamin C (260 mg/100 g) which is the second after amla (600 mg/100 g). It is a fair source of vitamin A and good source of calcium and phosphorus. It is rich in pectin. It's roots, bark, leaves and immature fruits, because of their astringency, are commonly employed to halt gastroenteritis, diarrhoea and dysentery. Guava fruits are relished when mature or ripe and can be processed into products like jam, juice, nectar and jelly. On the other hand, aloe vera is a popular medicinal plant. The virtues of aloe vera have been recorded for thousands of years by many ancient civilizations including Egypt, Persia, Greece, India and China. Among all the medicinal plants, aloe vera is one of the most important medicinal plants which has several therapeutic properties. World Health Organization (WHO) has given monographs on selected and widely used 28 medicinal plants to promote international harmonization in the quality control and use of herbal medicines. Among all 28 medicinal plants, aloe vera is one which has got the WHO monograph as a safer and healthier plant (WHO, 1999). It is popularly known as 'miracle plant'. Health benefits of aloe vera include increasing high density lipoproteins (HDL) level, reducing low density lipoproteins (LDL) level, reducing blood sugar in diabetics, effectiveness against AIDS and allergies and possess antiviral and antibacterial properties (Ahlawat and Khatkar, 2011). The reports on health benefits of aloe vera in health magazines and journals have encouraged urban populations to consume aloe vera gel. A common complaint by its consumers is its lesser enjoyable taste. Aloe vera gel can be incorporated in already popular and widely relished fruit products to ensure its enjoyable taste and wide spread utilization. Aloe vera is pectin deficient, while guava is pectin rich therefore the combination of both to prepare guava-aloe vera jelly will not only combine the medicinal value of aloe vera gel but will add the pleasing and aromatic taste of guava to the jelly. The present study was, therefore, undertaken to develop the aloe vera -guava jelly from different stages of aloe vera-guava pectin extracts, to study sensory attributes of final products, to analyze their proximate composition and to study the shelf life of the prepared jellies.

MATERIALS AND METHODS

The healthy disease free aloe vera leaves from four-year mature plants were harvested from aloe vera plantation raised at local herbal park. Four-year old

[1]Department of Entomology, CCS Haryana Agricultural University Hisar-125 004 (Haryana), India.

plant leaves were selected to recover highest gel yield (Ahlawat *et al.*, 2013a). The leaves were washed, trimmed, drained, filleted, homogenized and charcoal treated as per method of Ahlawat *et al.* (2013b) to extract fresh aloe vera gel. Sugar, citric acid and relevant materials required for the experiment were received from the department's store. Firm and ripe guava fruits were procured from local market of Hisar and were carefully chosen as the maturity stage affects the pectin content which is desirable for appropriate jelly formation. For preparation of aloe vera guava pectin extract, the washed guava fruits were cut into four quarters with a stainless steel knife. Guava pieces (1 kg) were boiled in 1 litre of aloe vera gel. Citric acid (1 g) was dissolved in small quantity of water and added to the mix during boiling. The mixture was boiled for half an hour in a stainless steel container. The juice was separated by filtering through a cheese cloth.

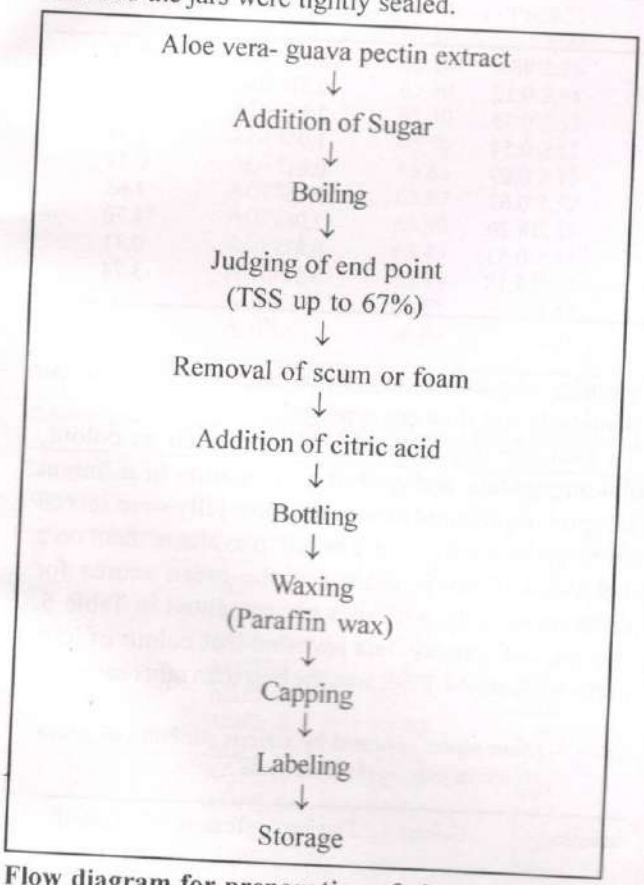


Flow diagram for preparation of Aloe vera-guava pectin extracts.

The three extracts of aloe vera-guava pectin were taken and mixed to develop five samples which were designated as per following scheme : first extract (AGPE-1), second extract (AGPE-2), third extract (AGPE-3), composite of first and second extracts (AGPE-4) and composite of all the three extracts (AGPE-5).

For preparation of jelly, 1 kg aloe vera-guava pectin extract was taken. Sugar quantity to be added was estimated by alcohol test. One teaspoonful of strained extract was taken in a beaker and cooled then three teaspoonfuls of methylated spirit was poured gently down the side of the beaker which was rotated for mixing and allowed to stand for a few minutes. An

equal amount of sugar was added to the extract for preparation of jelly when a single, transparent lump or clot was obtained suggesting that the extract was rich in pectin. When the clot formed was less firm and fragmented (i. e. medium pectin content), three-fourth of the amount of sugar was added. One-half of the amount of sugar was added to the extract poor in pectin as indicated by the formation of numerous small granular clots. Sugar based on pectin content was added and mixed thoroughly. The mixture was then boiled in a stainless steel container. The mixture was boiled and cooked till it gave the TSS equivalent to 67% and then citric acid 0.5 g dissolved in water was added near the end point. The finished product was poured into clear dry sterilized glass jars. The melted paraffin wax was poured in the jars just to cover the surface of jelly. Then the samples were allowed to cool and the jars were tightly sealed.



Flow diagram for preparation of aloe vera-guava jelly.

The prepared jelly samples were divided into two lots and stored at room temperature (30±2°C) and refrigerated temperature (4°C). The samples were drawn at an interval of every 30 days up to three months to observe the changes in pH, TSS, acidity, colour, flavour and signs of fungal growth.

The fresh aloe vera gel was analysed for its physico-chemical characteristics. Specific gravity and refractive index were measured at 20°C using pycnometer and an automatic refractometer, respectively. Viscosity was measured at 20°C with Brookfield viscometer using spindle number 2 at 60 rpm. Digital pH meter was used for pH determination. Titratable acidity (TA), total sugars (TS) and total solids were determined as per methods described by Ranganna (1986). The total soluble solids (TSS) were measured by Erma hand refractrometer of 0-32°B range. The bioactive principal, acemannan, content was estimated by method of McAnalley (1988). The fresh sample of guava, aloe vera-guava pectin extracts and their composites were analyzed for moisture, ash, vitamin C (ascorbic acid), total soluble solids, pH, titratable acidity, reducing sugar, non-reducing sugar and total sugar content as per the methods of Ranganna (1986). Aloe vera-guava jellies were analyzed for moisture, ash, total soluble solids, total sugars, titratable acidity, pH, vitamin C, protein, fat and fiber content as per the methods of Ranganna (1986).

The sensory attributes of developed guava-aloe vera jellies were evaluated by a sensory panel comprising 10 semi-trained panelists selected from the students, teachers and employees of the Department of Food Technology, GJUS & T, Hisar. The panelists were asked to assign appropriate score on a 1 to 9-Point Hedonic scale (Amerine *et al.,* 1965) for characteristic colour, flavour, texture and overall acceptability to the five samples of the developed guava-aloe vera jelly. The scale was arranged such that : 9=Like extremely, 8=Like very much, 7=Like moderately, 6=Like lightly, 5=Neither like nor dislike, 4=Dislike slightly, 3=Dislike moderately, 2=Dislike very much and 1=Dislike extremely.

Date represented are means of triplicates in case of physico-chemical parameters and mean of 10 values in case of sensory evaluation.

RESULTS AND DISCUSSION

Perusal of data presented in Table 1 indicates that fresh aloe vera gel had high percentage of water and low values for TSS and total sugar. It was acidic in nature. Data pertaining to raw guava pulp's moisture, TSS, ash, reducing sugar, non-reducing sugar, total sugar, ascorbic acid, acidity and pH are presented in Table 2. Composition of first extract (AGPE-1), second extract (AGPE-2), third extracts (AGPE-3), composite of first and second extract (AGPE-4) and composite of first, second and third extracts juice (AGPE-5) has been shown in Table 3.

Table 1. Physico-chemical characteristics of fresh aloe vera gel

Physico-chemical properties	Values
Specific gravity	1.0064±0.00
Refractive index	1.3348±0.00
Viscosity (cP)	52.70±0.29
pH	4.81±0.00
Titrable acidity as malic acid (%)	0.47±0.02
Total solids (%)	1.10±0.01
Total sugars (%)	0.27±0.01
Total soluble solids (°B)	1.00±0.00
Acemannan (%)	0.20±0.02

*Standard deviation for specific gravity and refractive index was almost zero.
Data represented are means of three values.

Table 2 Composition on raw guava

Constituents	Amount
Moisture (%)	84.4±0.20
Total soluble solids (%)	12.30±0.00
Ash (%)	0.51±0.01
Reducing sugar (%)	3.70±0.25
Non- reducing sugar (%)	4.35±0.02
Total sugar (%)	8.05±0.03
Ascorbic acid (mg/100 g)	91.5±0.45
Acidity (%)	0.94±0.09
pH	6.5±0.00

Data represented are means of three values.

Table 3. Composition of different aloe vera-guava pectin extract and composites used for jelly preparation

Constituents	Amounts in different extracts and their composites				
	AGPE-1	AGPE-2	AGPE-3	AGPE-4	AGPE-5
Moisture (%)	95.5	97.4	98.6	96.45	97.16
Ash (%)	0.35	0.28	0.12	0.31	0.25
Total soluble solids (%)	3.50	1.75	0.75	2.62	2.00
Reducing sugars (%)	2.75	1.15	0.54	1.95	1.48
Non-reducing sugars (%)	0.25	0.18	0.09	0.21	0.17
Total sugars (%)	3.00	1.33	0.63	2.16	1.65
Ascorbic acid (mg/100 g)	55.47	30.45	18.20	42.96	34.70
Acidity (%)	0.91	0.71	0.53	0.81	0.71
pH	3.46	3.58	4.18	3.52	3.74

Data represented are means of three values.

Table 4. Composition of guava-aloe vera jelly with different extractions and composites

Constituents	Amounts in jellies developed				
	AGPE -1	AGPE -2	AGPE -3	AGPE -4	AGPE -5
Moisture (%)	22.22	22.45	22.6	21.53	22.0
Ash (%)	0.29	0.26	0.20	0.28	0.30
Total soluble solids (%)	66.00	65.5	65.0	67.0	66.0
Total sugars (%)	63.40	64.2	64.4	64.0	63.2
pH	3.03	3.0	2.9	3.2	3.0
Acidity (%)	0.73	0.65	0.7	0.68	0.74

AGPE-1–Aloe vera-guava jelly prepared by first extract, AGPE-2–Aloe vera-guava jelly prepared by second extract, AGPE-3–Aloe vera-guava jelly prepared by third extract, AGPE-4–Aloe vera-guava jelly prepared by composite of first and second extracts and AGPE-5–Aloe vera-guava jelly prepared by composite of three extracts.
Data represented are means of three values.

The composition of different samples of aloe vera-guava jellies has been shown in Table 4. The contents of protein, fat, fiber and vitamin C content of different samples of guava-aloe vera jellies were found to be negligible and thus not reported.

To evaluate the sensory attributes such as colour, flavour, texture and overall acceptability of different samples of prepared aloe vera-guava jelly were served to the panelists who were asked to evaluate them on a 1-9-Point Hedonic scale and the mean scores for different sensory attributes are presented in Table 5. Analysis of sensory data revealed that colour of jelly prepared from AGPE-2 was the best than other samples.

Table 5. Mean scores obtained by various attributes of guava-aloe vera jelly on Hedonic scale

Sample	Colour	Flavour	Texture	Overall acceptability
AGPE-1	7.80	8.10	7.30	7.80
AGPE-2	8.10	7.40	7.10	7.60
AGPE-3	7.10	6.60	7.40	7.10
AGPE-4	8.00	7.90	7.50	8.20
AGPE-5	7.30	7.20	8.00	7.50

Abbreviation details in first column are given in Table 4.
Data represented are means of 10 values.

AGPE-2 jelly got highest score i. e. 8.10, while AGPE-3 obtained the lowest score of 7.10. The flavour score was highest for AGPE-1 (8.10) and lowest for AGPE-3 (6.60). In case of texture, jelly prepared from AGPE-5 was rated best among other samples. AGPE-5 got highest score (8.00) and AGPE-2 the lowest score (7.10). Further on the basis of overall acceptability score, it was observed that the aloe vera-guava jelly prepared from AGPE-4 i. e. composite of first and second extracts was the best followed by those prepared from AGPE-1, AGPE-2, AGPE-5 and AGPE-3.

During storage, the changes in TSS, acidity, pH, colour, flavour and fungal growth of guava-aloe vera jellies were observed for 90 days after an interval of

Table 6. Observation on the storage stability of aloe vera-guava jelly samples stored at refrigeration temperature (4°C) for 90 days

Storage period (days)	Samples	TSS (°B)	pH	Acidity	Colour	Flavour	Fungal growth
0	AGPE-1	66.0	3.03	0.73	Light golden	Pleasant	Not visible
	AGPE-2	65.5	3.00	0.65	Light golden	Pleasant	Not visible
	AGPE-3	65.0	2.90	0.70	Light golden	Pleasant	Not visible
	AGPE-4	67.0	3.20	0.68	Light golden	Pleasant	Not visible
	AGPE-5	66.0	3.00	0.74	Light golden	Pleasant	Not visible
30	AGPE-1	66.15	3.00	0.73	Light golden	Pleasant	Not visible
	AGPE-2	65.30	2.80	0.65	Light golden	Pleasant	Not visible
	AGPE-3	64.95	2.72	0.70	Light golden	Pleasant	Not visible
	AGPE-4	67.10	3.00	0.68	Light golden	Pleasant	Not visible
	AGPE-5	66.14	2.76	0.74	Light golden	Pleasant	Not visible
60	AGPE-1	65.90	2.64	0.95	Light golden	Pleasant	Not visible
	AGPE-2	65.30	2.53	0.84	Light golden	Pleasant	Not visible
	AGPE-3	65.70	2.51	0.88	Light golden	Pleasant	Not visible
	AGPE-4	66.85	2.75	0.78	Light golden	Pleasant	Not visible
	AGPE-5	65.87	2.52	0.86	Light golden	Pleasant	Not visible
90	AGPE-1	65.90	2.35	1.25	Light golden	Pleasant	Not visible
	AGPE-2	65.45	2.34	1.15	Light golden	Pleasant	Not visible
	AGPE-3	65.75	2.31	1.21	Light golden	Pleasant	Not visible
	AGPE-4	66.85	2.42	1.09	Light golden	Pleasant	Not visible
	AGPE-5	66.90	2.29	1.26	Light golden	Pleasant	Not visible

Abbreviation details in second column are given in Table 4.

Table 7. Observation on the storage stability of aloe vera-guava jelly samples stored at ambient temperature (30±2°C) for 90 days

Storage period (days)	Samples	TSS (°B)	pH	Acidity	Colour	Flavour	Fungal growth
0	AGPE-1	66.0	3.03	0.73	Light golden	Pleasant	Not visible
	AGPE-2	65.5	3.00	0.65	Light golden	Pleasant	Not visible
	AGPE-3	65.0	2.90	0.70	Light golden	Pleasant	Not visible
	AGPE-4	67.0	3.20	0.68	Light golden	Pleasant	Not visible
	AGPE-5	66.0	3.00	0.74	Light golden	Pleasant	Not visible
30	AGPE-1	65.20	2.72	0.81	Light golden	Pleasant	Not visible
	AGPE-2	65.40	2.70	0.75	Light golden	Pleasant	Not visible
	AGPE-3	64.90	2.58	0.79	Light golden	Pleasant	Not visible
	AGPE-4	66.43	2.83	0.70	Light golden	Pleasant	Not visible
	AGPE-5	66.20	2.62	0.80	Light golden	Pleasant	Not visible
60	AGPE-1	65.80	2.50	1.02	Light golden	Pleasant	Not visible
	AGPE-2	65.50	2.45	0.92	Light golden	Pleasant	Not visible
	AGPE-3	64.85	2.43	0.95	Light golden	Pleasant	Not visible
	AGPE-4	66.56	2.61	0.85	Light golden	Pleasant	Not visible
	AGPE-5	66.30	2.47	0.93	Light golden	Pleasant	Not visible
90	AGPE-1	65.90	2.28	1.28	Light golden	Pleasant	Not visible
	AGPE-2	65.60	2.26	1.25	Light golden	Pleasant	Not visible
	AGPE-3	64.90	2.25	1.18	Light golden	Pleasant	Not visible
	AGPE-4	66.50	2.32	1.13	Light golden	Pleasant	Not visible
	AGPE-5	66.25	2.23	1.30	Light golden	Pleasant	Not visible

Abbreviation details in second column are given in Table 4.

30 days at refrigerated storage i. e. 4°C (Table 6) and ambient temperature i. e. 30±2°C (Table 7). It was observed that the TSS of jellies did not show any remarkable change up to 90 days of storage under both the conditions. pH showed an overall decrease during storage and acidity exhibited a corresponding increase during the storage period of 90 days. No remarkable changes in colour and flavour were observed up to 90 days of storage. Also no fungal growth was observed during storage period under both the storage conditions.

CONCLUSIONS

This study indicated a bright prospect of processing of jelly from aloe vera-guava pectin extracts. Aloe vera-guava jelly provides an easy method of incorporation of a highly important medicinal plant in an enjoyable fruit product making it a functional food product. The jelly prepared using aloe vera-guava pectin extract and their composites were acceptable for consumption but the jelly prepared with a composite of first and second extracts juice was found most promising on the basis of sensory data. The prepared guava-aloe vera jelly samples were shelf stable up to a period of 90 days under storage at ambient as well as refrigerated temperature.

REFERENCES

Ahlawat, K. S. and Khatkar, B. S. (2011). Processing, food applications and safety of aloe vera products : a review. *J. Food Sci. Technol.* **48** : 525-533.

Ahlawat, K. S., Khatkar, B. S., Sushil and Gulia, N. (2013b). Effect of processing and storage on sensory properties and acemannen content of aloe vera gel. *Annals of Agri-Bio Res.* **18** : 70-73.

Ahlawat, K. S., Sushil, Gulia, N. and Khatkar, B. S. (2013a). Effect of plant maturity on leaf growth, yield and physico-chemical properties of aloe vera gel. *Agro Food Ind. Hi-Tech.* **24** : 35-37.

Amerine, M. A., Pangborn, R. M. and Roessler, E. B. (1965). *Laboratory Studies : Quantity-quality Evaluation.* Academic Press, New York. pp. 349-397.

McAnalley, B. H. (1988). Process for preparation of aloe products produced thereby and composition thereof. *US Patent* **4** : 735,935.

Ranganna, S. (1986). *Handbook of Analysis and Quality Control for Fruit and Vegetable Products.* Tata McGraw Hill, New Delhi.

WHO (1999). *WHO Monographs on Selected Medicinal Plants* **1** : 289.

Annals of Agri-Bio Research **18** (1) : 70-73, 2013

Effect of Processing and Storage on Sensory Properties and Acemannan Content of Aloe Vera Gel

KULVEER SINGH AHLAWAT, B. S. KHATKAR*, SUSHIL[1] AND NEELAM GULIA

Department of Food Technology, Guru Jambheshwar University of Science & Technology, Hisar-125 001 (Haryana) India

****(e-mail : bhup2009@hotmail.com)***

ABSTRACT

Aloe vera gel was extracted from healthy disease free leaves of 4 years old mature plants. A process technology with respect to draining time of yellow exudates from aloe vera leaves, charcoal treatment time, degree of heat treatment and amount of xanthan gum added to fresh aloe vera gel was standardized to produce an acceptable stabilized aloe vera gel possessing improved sensory attributes and having bioactive principle (acemannan) in amounts comparable to fresh aloe vera gel. Results demonstrated that draining the yellow exudates from leaves for 90 min, charcoal treatment of 90 min, heat treatment of 85°C for 30 min and addition of xanthan gum at 0.5% (w/w) to fresh aloe vera gel was found appropriate to produce acceptable stabilized aloe vera gel. The stabilized aloe vera gel processed by the standardized method was studied for changes in its sensory attributes and acemannan content over a storage period of 90 days at ambient temperature (30°C±2°C). Results demonstrated that the stabilized aloe vera gel had appealing colour, taste, flavour and appearance, superior to that of fresh aloe vera gel and at the same time had acemannan content comparable to fresh gel over the storage period.

Key words : Aloe vera gel, charcoal treatment, stabilization, yellow exudates, acemannan

INTRODUCTION

The parenchyma cells of aloe vera leaf contain a transparent mucilaginous jelly which is termed as aloe vera gel (Femenia *et al.,* 1999). It has long been used in health foods, medicines and for cosmetic purposes (Grindlay and Reynolds, 1986). A prominent feature of aloe vera gel is its high water content of about 99% of fresh matter. More than 60% of the remaining solid is made up of polysaccharides with minor amounts of various other components (McAnalley, 1993). Acemannan, a mannose containing polysaccharide, has been reported as the main bioactive substance present in aloe vera gel (t'Harat *et al.,* 1989; McAnalley, 1993) and held responsible for majority of therapeutic benefits of aloe vera gel. Acemannan is reported to accelerate wound healing, modulate immune functions (particularly macrophase activation and production of cytokines) and demonstrates antineoplastic and antiviral effects (Peng *et al.,* 1991; Zang and Tizard, 1996; Ramamoorthy *et al.,* 1996). Acemannan has demonstrated reduction of skin damage following exposure to gamma radiations (Robert and Travis, 1995). Acemannan has also shown to increase the response of lymphocytes to antigens in an *in vitro* study (Womble and Helderman, 1988). Some studies confirm the anti-cancerous effects of acemannan (Peng *et al.,* 1991). Antifungal activity, anti-diabetic effects, anti-inflammatory, gastroprotective properties and their effectivity in treatment of AIDS are also attributed to acemannan (Hamman, 2008). Acemannan has also been shown to inhibit cholesterol absorption in intestine thereby lowering of blood cholesterol level. The fresh aloe vera gel is edible but does not possess enjoyable taste and odour mainly due to contamination with the yellow exudates present in bundle sheath cells beneath the green rind of aloe leaf. The gel often gets contaminated with yellow exudates during the imperfect extraction procedures. Fresh aloe vera gel is reported to be very unstable as it rapidly oxidizes, decomposes, and looses much of its biological activities (Coats and Ahola, 1979) and develops nauseating off odour, ultimately separating into two distinct layers lower one of settled solids and upper one of transparent watery portion with gas bubbles on surface i. e. the gel get destabilized. The colour, aesthetic appeal and therapeutic effects of fresh aloe vera gel diminish with destabilization. The decomposition (destabilization) of the gel begins just after removal of leaf from plant due to native enzymes (enzymes clip acemannan chains), growth of bacteria within the gel and chemical reactions, acetyl group from acemannan is liberated (Eshun and He, 2004). The sensory attributes and the biological activities of fresh aloe vera gel must be preserved for extended periods after its extraction for its effective commercial exploitation in food, pharma and cosmetic applications.

[1]Department of Applied Sciences, Govt. Polytechnic, Hisar-125 001 (Haryana), India.

The process of preserving the sensory and biological activities of extracted fresh gel for extended periods is called stabilization. Some workers have attempted to stabilize aloe vera gel and delay its degradation by admixture with natural polysaccharides (Yaron *et al.*, 1992). Some other juices are also made shelf stable with various additives e. g. carrageenans have been used to inhibit browning of fresh apple juice (Tong and Hicks, 1991) and cyclodextrins to inhibit aggregation of β-casein (Lee and Fennema, 1991). However, scanty information is available about the effective technique to produce a stabilized and shelf stable aloe vera gel having acemannan content in quantities comparable to that of fresh aloe vera gel and having enjoyable sensory attributes. The present study, therefore, was undertaken with two objectives. Firstly to optimize process variables for improving sensorial quality and preserving the bioactive principle of aloe vera gel i. e. to produce a stabilized aloe vera gel and secondly to undertake storage studies of this stabilized gel.

MATERIALS AND METHODS

Mature outer leaves from disease free 4-year old aloe vera plants were procured from local herbal park, Hisar. Leaves were washed in double distilled water and the basal 1½ inch white portion of the washed leaves was removed with a sharp knife and the trimmed leaves were divided into two lots, smaller consisting of three leaves and larger one of 10 leaves. Each leaf of smaller lot was kept with the broader end facing ground in a separate container to drain yellow exudate for 30, 60 and 90 min, respectively. After draining, the leaves were divided into strips of about 1 inch width and the green rind from each leaf strip was removed by inserting knife up to 2 mm deep into parenchyma tissue below the outer rind and proceeding length-wise to remove the green rind for extraction of gel filet. Later it was homogenized in Waring blender of 1 l capacity at high speed for 2 min. The homogenized mixture was filtered through muslin cloth to get fresh aloe vera gel. The larger lot of trimmed leaves was processed to fresh aloe vera gel without draining the yellow exudate and divided into 10 portions. One served as control and remaining nine portions were subjected to different processing treatments. Three portions were given charcoal treatment for 30, 60 and 90 min. Other three portions of fresh aloe vera gel were subjected to in-bottle heat treatment at 65°C 30 min, 75°C 30 min and 85°C 30 min. To the remaining three portions xanthan gum was added at 0.25, 0.50 and 0.75% levels. The treatment options rated best in terms of sensory quality and acemannan content were selected and combined to achieve the stabilization of fresh aloe vera gel. Sodium bezoate at level of 750 ppm was added to stabilized aloe vera gel and packed in 200 ml pre-sterilized glass bottles. The samples followed by labelling were stored for three months at ambient temperature 30°C±2°C and analyzed after every 30 days interval for sensory attributes and acemannan content (Table 3).

The fresh aloe vera gel was analysed for its phsyco-chemical characteristics. Specific gravity and refractive index were measured at 20°C using pycnometer and an automatic refractometer, respectively. Viscosity was measured at 20°C with Brookfield viscometer using spindle number 2 at 60 rpm. Digital pH meter was used for pH determination. Titratable acidity (TA) and total sugars (TS) were determined as per methods described by Ranganna (1986). The total soluble solids (TSS) were measured by Erma hand refractrometer of 0-32°Brix. The bioactive principle, acemannan, content was estimated by method of McAnalley (1988). Data are given in Table 1.

Table 1. Physico-chemical characteristics of fresh aloe vera gel

Physico-chemical properties	Value*
Specific gravity	1.0064±0.00
Refractive index	1.3348±0.00
Viscosity (cP)	52.70±0.29
pH	4.81±0.00
Titrable acidity as malic acid (%)	0.47±0.02
Total sugars (%)	0.27±0.01
Total soluble solids (°B)	0.10±0.00
Acemannan (%)	0.20±0.02

*Standard deviation for specific gravity and refractive index was almost zero.

All the samples i. e. control as well as those derived from each variation of every treatment were rated on 9-point Hedonic scale (Amerine *et al.*, 1965) for colour, flavour, taste, appearance (homogeneous or signs of settling of suspended solids) and overall acceptance by a panel of 10 semi-trained judges to select the best rated sample for finding optimum degree of variation of each treatment at the time of preparation (Table 2). The observations for appearance were made after interval of 12 h to examine signs of settling of solids. The aloe vera gel prepared by combining the best treatment options i. e. the stabilized aloe vera gel was analysed for sensory attributes as well as acemannan content on preparation day and after every 30 days for a period of three months.

All determinations were made in triplicate. Results were analyzed by one-way analysis of variance. The mean comparison was carried out with Duncan's multiple range test employing SPSS software version 16.0 (SPSS Inc.) The statistical significance was observed at $P<0.05$.

RESULTS AND DISCUSSION

The physico-chemical composition presented in Table 1 reveals that fresh aloe vera juice contained very low amount of total sugars, total soluble solids and titrable acidity. Femenia *et al.* (1999) reported similar values for various physico-chemical characteristics of fresh aloe vera gel derived from 4-year old plants.

Data in Table 2 depict sensory evaluation score of different quality attributes of aloe vera gel obtained by variations in processing methodology as well as of control. The score for colour improved significantly as draining time of yellow exudates were increased from 30 to 90 min. It can be concluded that more of yellow exudates were eliminated from the leaf with passage of time leaving very low amount of it in the leaf that may find a chance to contaminate the gel during extraction process. Increase in charcoal treatment duration from 30 to 90 min also improved the colour score up to 7.8 against 4.4 for control. Yellow colour imparted by the residual exudates was absorbed by charcoal with increased treatment duration. Increase in degree of heat treatment had a positive effect on colour might be due to greater thermal degradation of colour imparting exudate. Xanthan gum at level of 0.50% improved the colour most effectively. Taste score was noted the highest for draining time of 90 min among the three variations of this unit operation because of efficient removal of bitter yellow exudates from the leaf. Score for taste was the highest for charcoal treatment of 90 min might be due to efficient absorption of bitter compounds of gel. Taste score was noted the highest for heat treatment of 85°C for 30 min among all the three variations of the degree of this treatment probably due to destruction of bitter tasting compounds of gel. Xanthan gum of 0.5% level was again found most appropriate among the three levels for taste also.

Table 2. Effect of processing treatments on sensory characteristics and acemannan content of aloe vera gel

Treatment	Colour*	Taste*	Flavour*	Appearance*	Overall acceptability*	Acemannan (%)
Control	4.4 ± 0.5^{a}	2.3 ± 0.5^{a}	3.3 ± 0.5^{a}	4.2 ± 0.4^{a}	4.3 ± 0.5^{a}	0.20 ± 0.02^{a}
Draining time (30 min)	5.1 ± 0.4^{c}	4.7 ± 0.5^{b}	5.2 ± 0.4^{c}	4.4 ± 0.5^{ab}	5.1 ± 0.4^{c}	0.20 ± 0.02^{a}
Draining time (60 min)	6.1 ± 0.4^{de}	6.2 ± 0.6^{e}	6.1 ± 0.6^{e}	4.5 ± 0.6^{ab}	5.8 ± 0.4^{d}	0.20 ± 0.02^{a}
Draining time (90 min)	6.8 ± 0.4^{g}	6.7 ± 0.5^{f}	6.7 ± 0.5^{f}	4.5 ± 0.6^{ab}	7.3 ± 0.5^{f}	0.20 ± 0.02^{a}
Charcoal treatment (30 min)	6.3 ± 0.5^{ef}	5.2 ± 0.4^{c}	6.3 ± 0.5^{e}	4.2 ± 0.4^{a}	5.8 ± 0.4^{d}	0.20 ± 0.02^{a}
Charcoal treatment (60 min)	7.1 ± 0.4^{h}	5.8 ± 0.4^{d}	6.8 ± 0.4^{f}	4.1 ± 0.4^{a}	6.9 ± 0.4^{e}	0.20 ± 0.02^{a}
Charcoal treatment (90 min)	7.8 ± 0.4^{i}	6.8 ± 0.4^{f}	7.7 ± 0.5^{g}	4.2 ± 0.4^{a}	7.8 ± 0.4^{g}	0.20 ± 0.02^{a}
Pasteurization (65°C, 30 min)	4.8 ± 0.4^{b}	5.7 ± 0.5^{d}	4.7 ± 0.5^{b}	4.9 ± 0.4^{cd}	4.7 ± 0.5^{b}	0.18 ± 0.01^{b}
Pasteurization (75°C, 30 min)	5.9 ± 0.4^{d}	6.2 ± 0.4^{e}	6.1 ± 0.3^{e}	6.3 ± 0.5^{f}	5.9 ± 0.4^{d}	0.18 ± 0.01^{b}
Pasteurization (85°C, 30 min)	6.8 ± 0.4^{g}	6.7 ± 0.5^{f}	6.7 ± 0.5^{f}	7.3 ± 0.5^{g}	7.3 ± 0.5^{f}	0.18 ± 0.01^{b}
Xanthan gum (0.25%)	5.2 ± 0.4^{c}	5.1 ± 0.4^{c}	5.3 ± 0.5^{c}	5.9 ± 0.4^{e}	5.8 ± 0.4^{d}	0.20 ± 0.02^{a}
Xanthan gum (0.50%)	5.8 ± 0.4^{d}	5.7 ± 0.5^{d}	5.7 ± 0.5^{d}	7.8 ± 0.4^{h}	7.9 ± 0.4^{g}	0.20 ± 0.02^{a}
Xanthan gum (0.75%)	4.8 ± 0.4^{b}	4.6 ± 0.5^{b}	4.9 ± 0.4^{b}	4.7 ± 0.5^{bc}	4.8 ± 0.4^{b}	0.20 ± 0.02^{a}

*Mean±standard deviation in the same column followed by different superscript differ significantly at $P<0.05$; Rating on 9-point Hedonic scale, 9=Like extremely, 1=Dislike extremely.

The highest score for flavour, appearance and overall acceptability was obtained when aloe vera gel was processed for 90 min draining time, charcoal treatment of 90 min, pasteurization at 85°C for 30 min and xanthan gum level at 0.05%.

The acemannan content was not affected by variations of draining time, charcoal and level of xanthan gum addition. It showed a slight decrease as heat treatment was increased but decrease was not drastic and the amount of acemannan left after treatment was comparable to that of fresh aloe vera gel. The decrease in acemannan content might be due to thermal degradation (Chang *et al.*, 2006). The heat treatment at 85°C for 30 min was selected as optimum as score for sensory attributes was higher at this processing condition.

The score for all the sensory attributes attained the highest values in case of draining time of 90 min, charcoal treatment of 90 min, pasteurization at 85°C for 30 min and addition of xanthan gum at level of 0.5% clearly establishing these as the most effective degrees of the treatments. It was also inferred from perusal of data contained in Table 2 that the aloe vera

gel obtained after encountering every variation of the four treatments scored significantly higher for colour, taste, flavour, appearance and overall acceptability than the control. The analysis of the stabilized aloe vera gel prepared by combining all the four most effective treatment options on preparation day revealed significantly higher scores for sensory attributes over the control. The acemannan showed a slight decrease compared with control. Changes in these parameters were small over the storage period of 90 days. This clearly demonstrated that stabilized aloe vera gel was self stable over the storage period studied (Table 3).

Table 3. Effect of storage intervals on sensory properties and acemannan content of stabilized aloe vera gel

Sensory characteristics	Storage period (days)			
	0	30	60	90
Colour	6.33	6.27	6.20	6.13
Taste	6.30	6.39	6.45	6.52
Flavour	6.25	6.23	6.21	6.20
Appearance	6.30	6.29	6.27	6.25
Overall acceptability	6.25	6.20	6.18	6.15
Acemannan (%)	0.18	0.18	0.18	0.17

CONCLUSIONS

The aloe vera gel processed by draining of yellow exudate from the leaves for 90 min, giving the charcoal treatment for 90 min to the gel, pasteurizing the gel at 85°C for 30 min and addition of xanthan gum at level of 0.5% was found to be a stable product. The stabilized aloe vera gel resulted from the standardized process and was much superior than the fresh aloe vera gel in terms of organoleptic quality and it was comparable to fresh gel in terms of content of bioactive principle (acemannan). The resulted stabilized aloe vera gel was self stable up to 90 days of storage period at ambient conditions.

REFERENCES

Amerine, M. A., Pangborn, R. M. and Roessler, E. B. (1965). *Laboratory Studies : Quantity-Quality Evaluation.* Academic Press, New York. pp. 349-397.

Chang, X. L., Wang, C., Feng, Y. and Liu, Z. (2006). Effects of heat treatments on the stabilities of polysaccharides substances and barbaloin in gel juice from aloe vera Miller. *J. Food Engg.* **75** : 245-251.

Coats, B. C. and Ahola, R. (1979). Aloe vera–the silent healer. In : *A Modern Study of Aloe Vera. Dallas, Vol. 1,* Caots, B. C. (ed.) pp. 288-294.

Eshun, K. and He, Q. (2004). Aloe vera : A valuable ingredient for the food, pharmaceutical and cosmetic industries : A review. *Crit. Rev. Food Sci. Nutr.* **44** : 91-96.

Femenia, A., Sanchez, E. S., Simal, S. and Rossello, C. (1999). Compositional features of polysaccharides from Aloe vera (*Aloe barbadensis* Miller) plant tissues. *Carbohydrate Polymer* **39** : 109-117.

Grindlay, D. and Reynolds, T. (1986). The aloe vera phenomenon : a review of the properties and modern uses of the leaf parenchyma gel. *J. Ethnopharmacol.* **16** : 117-151.

Hamman, J. H. (2008). Composition and applications of aloe vera leaf gel. *Molecules* **13** : 1599-1616.

Lee, M. J. and Fennema, O. R. (1991). Ability of cyclodextrins to inhibit aggregation of β-casein. *J. Agri. Food Chem.* **39** : 17-21.

McAnalley, B. H. (1988). Process for preparation of aloe products produced thereby and compositions thereof. *US Patent* **4** : 735-935.

McAnalley, B. H. (1993). Process for preparation of aloe products. European Patent WO 89/06539.

Peng, S. Y., Norman, J., Cutin, G., Corrier, D., McDaniel, H. R. and Busbee, D. (1991). Decreased mortality of Norman murine sarcoma in mice treated with the immunomodulator, acemannan. *Mol. Biother.* **3** : 79-87.

Ramamoorthy, L., Kemp, M. C. and Tizard, I. R. (1996). Acemannan, a beta-(1,4)-acetylated mannan, induces nitric oxide production in macrophage cell line RAW 264.7. *Mol. Pharmocol.* **50** : 878-884.

Ranganna, S. (1986). *Handbook of Analysis and Quality Control for Fruit and Vegetable Products.* Tata McGraw Hill, New Delhi.

Robert, O. and Travis, E. (1995). Acemannan containing wound dressing gel reduces radiation-induced skin reactions in C3H mice. *Int. J. Radiat. Oncol. Biol. Phys.* **32** : 1047-1052.

t'Hart, I. A., van den Berg, A. J., Kuis, L., van Dijk, H. and Lanbadie, R. P. (1989). An anti-complementary polysaccharide with immunological adjuvant activity from the leaf parenchyma gel of aloe vera. *Plant Med.* **55** : 509-512.

Tong, C. B. S. and Hicks, K. B. (1991). Sulfated polysaccharides inhibit browning of apple juice and diced apples. *J. Agric. Food Chem.* **39** : 1719-1722.

Womble, D. and Helderman (1988). Enhancement of allo-responsiveness of human lymphocytes by acemannan (Carrisyn). *Int. J. Immunopharmacol.* **10** : 957-974.

Yaron, A., Cohen, E. and Arad, S. M. (1992). Stabilization of aloe vera gel by interaction with sulfated polysaccharides from micro algae and with xantham gum. *J. Agric. Food Chem.* **40** : 1316-1340.

Zang, L. and Tizard, I. R. (1996). Activation of a mouse macrophase cell line by acemannan, the major carbohydrate fraction from aloe vera gel. *Immunopharmacol.* **35** : 119-128.

J Food Sci Technol (September–October 2011) 48(5):534–541
DOI 10.1007/s13197-010-0225-8

REVIEW ARTICLE

Trans fats—sources, health risks and alternative approach - A review

Vandana Dhaka · Neelam Gulia · Kulveer Singh Ahlawat · Bhupender Singh Khatkar

Revised: 10 November 2010 / Accepted: 29 November 2010 / Published online: 28 January 2011

Abstract Trans fatty acids have the presence of one or more double bonds in the trans configuration instead of the usual cis configuration. They are desired by Vanaspati industry as they impart firmness to margarines and plasticity as well as emulsion stability to shortenings. Research has proved the direct connection of trans fatty acids with cardiovascular diseases, breast cancer, shortening of pregnancy period, risks of preeclampsia, disorders of nervous system and vision in infants, colon cancer, diabetes, obesity and allergy. In light of these new findings trans fatty intake should be zero and new technology of hydrogenation of oils is to be developed which produce zero trans fatty acids at the same time preserve the desirable properties contributed by trans fatty acids to the hydrogenated oils. Presently in India there is no system to monitor and regulate the amount of trans fats in processed foods and hence a stringent food law is immediately required.

Keywords Trans fatty acids · Hydrogenation · Interesterification · Trait-enhanced oils · Low density lipoproteins

Introduction

Fat intake excess of 35% of daily calorie requirements are associated with both total increased saturated fat and calorie intakes. Trans fat increases low density lipoproteins (LDL), triglycerides and insulin levels and reduces beneficial high density lipoproteins (HDL). The overall picture of trans fatty acids (TFA) implies a detrimental effect of TFA on health. However, due to the potential isomer specific effects of TFA, a blanket statement cannot be applied to the wide variety of TFA. Processed food industry has an important role in decreasing trans fatty acid content of the food supply by using the alternatives sources of fat with zero TFA level in the processed foods.

All natural fats and oils are a combination of monounsaturated, polyunsaturated and saturated fatty acids. Trans fatty acids (TFAs) are unsaturated fatty acids that contain at least one double bond in the trans configuration (Fig. 1). Trans fatty acids are formed during industrial partial hydrogenation of vegetable oil, a process widely commercialized to produce solid fats. The TFA content of partially hydrogenated vegetable oils (PHVO) depends on the variables of the hydrogenation process i.e. time, catalyst, temperature, and hydrogen pressure; the types and proportions of oils and composition of monounsaturated fatty acids (MUFA) and poly unsaturated fatty acids (PUFA) (Ghafoorunissa 2008).

Sources of trans fatty acids

Dietary fatty acids with trans double bonds come primarily from Industrial sources i.e. by partial hydrogenation of edible oils containing unsaturated fatty acids to saturated fats and secondly from bacterial transformation of unsaturated fatty acids in the rumen of ruminants. Ruminant and industrial fats contain the same TFA isomers, but the proportions differ (Weiland et al. 1999). The primary dietary TFA are vaccenic acid and elaidic acid. Vaccenic acid (18:1, trans-11) is the major ruminant TFA, whereas elaidic acid (18:1, trans-9) is the main TFA isomer in industrial hydrogenation (Mensink 2005; Weiland et al. 1999). The trans fatty acid content of industrially hydrogenated fats varies widely and may account for up to 60% of the fatty acid content, whereas the trans fatty acid content of beef and dairy products is considerably

V. Dhaka · N. Gulia · K. S. Ahlawat · B. S. Khatkar (✉)
Department of Food Technology,
Guru Jambheshwar University of Science and Technology,
Hisar, India 125001
e-mail: bskhatkar@yahoo.co.in

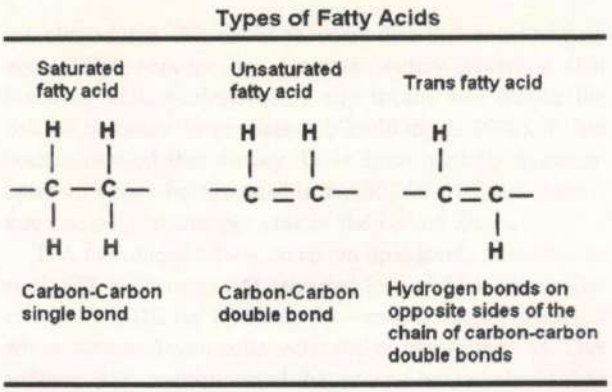


Fig. 1 Types of fatty acids showing trans configuration

lower and accounts for 2%–5% of the fatty acid content (Weggemans et al. 2004). In the case of special dietary choices, this allows for a daily intake of up to 10 times more industrially produced trans fatty acids than trans fatty acids from ruminants. Processed foods and oils provide approximately 80% of trans fats in the diet, compared to 20% that occur naturally in food from animal sources. The dietary intake of trans fats in some countries is depicted in Table 1. The major dietary sources of trans fats are cakes, cookies, crackers, animal products, margarine, fried potatoes, potato chips, popcorn and household shortening (Table 2). Limited consumption of foods made with processed sources of trans fats provides the most effective means of reducing intake of trans fats. To meet the recommended dietary intake for fat i.e. amount corresponding to 20 to 35% of calories, most dietary fats should come from sources of polyunsaturated and monounsaturated fatty acids. Plant sources of polyunsaturated

Table 1 Intake of industrially produced trans-fatty acids in different countries

Country	Ruminant (%)	Industrially produced TFA (%)			Mixed (%)	% Total energy
		Total	Fast foods	Spreads		
Australia	60	24	8–24	16		0.6
NZ	41	46	3–16		13	0.7
Canada	19	81	22	37		2.2
Denmark	50	50				1
US	21	79	–	17		2.6
Europe						0.9
Europe						1–2
UK				18		1.2
Iran						4.2
India[a]						0.9–1.35

[a] Risk Assessment Report on TFAs in Indian Diets submitted by National Institute of Nutrition, Hyderabad in October, 2009

Source: Skeaff 2009

Table 2 Contribution of various foods to trans fat intake in the diet

Food group	Contribution (per cent of total trans fats consumed)
Cakes, cookies, crackers, bread etc.	40
Animal products	21
Margarine	7
Fried potatoes	8
Potato chips, corn chips, popcorn	5
Household shortening	4
Breakfast cereals and candy etc.	5

fatty acids are vegetable oils, including soybean oil, corn oil, canola oil, walnuts, flaxseed and safflower oil. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are omega 3 fatty acids that are contained in fish and shellfish. Plant sources that are rich in monounsaturated fatty acids include canola oil, olive oil, high oleic safflower oil, and sunflower oil and nuts.

Trans fats are not formed during frying process. Tsuzuki (2010) has reported that an ordinary frying process using unhydrogenated oils has little impact on TFAs intake from edible oils. No trans fatty acids were formed in unhydrogenated and hydrogenated soyabean oil during heating at 160, 180 or 200 °C for 24 h, implying that trans fatty acid can only be formed under drastic heating conditions i.e. heating the oil at high temperatures or reusing the oil many times (Liu et al. 2007).

Health risks of trans fatty acids

A low intake of fats and oils (less than amount corresponding to 20% of daily calorie intake) increases the risk of inadequate intakes of vitamin E and of essential fatty acids and may contribute to unfavourable changes in HDL and triglycerides. Following risks are reported to be associated with the intake of trans fatty acids on human health.

Cardiovascular diseases Many years of epidemiological research have shown that populations consuming diets high in saturated fatty acids show relatively high levels of serum cholesterol and carry a high prevalence of coronary heart disease (Caggiula and Mustad 1997; Kromhout and Lezenne 1984; Keys 1980). Based on the evidence of various studies conducted, it is generally accepted that high levels of serum cholesterol, particularly LDL, promote the development of atherosclerosis or coronary heart disease. Mensink and Katan (1990) suggested that trans fats increased LDL and decreased the beneficial HDL resulting in a less desirable LDL/HDL ratio. Invariably, it was

established that TFA could be worse than the saturated fatty acids. The concept has become widely accepted that lowering LDL cholesterol by any means will reduce the risk of coronary heart disease (Gould et al. 1998). It has been estimated that dietary TFAs from partially hydrogenated oils may be responsible for 30,000–100,000 premature coronary deaths per year in the United States.

TFA has unique effects on serum lipid levels. Mozaffarian et al. (2006) reported that saturated fat and TFA had similar effects on LDL on a calorie for—calorie basis. However, when compared with either saturated or unsaturated fat, TFA reduced HDL and increased the ratio of total cholesterol to HDL. TFA consumption also increased serum triglyceride and lipoprotein levels and reduced LDL particle size in controlled trials indicating higher risk of coronary heart disease. These adverse effects of trans fatty acids have been confirmed by subsequent metabolic studies (Aro et al. 1997; Judd et al. 1994; Lichtenstein et al. 1999; Zock et al. 1995). Williams et al. (1998) established an association between TFA and incidence of non-fatal myocardial infarction from coronary heart disease. Relative risk for cardiovascular disease was increased by 27% as a result of consumption of TFA (Ascherio et al. 1994). Although it is established that TFAs increase LDL levels and decrease HDL levels (markers of coronary heart disease), little is known about the mechanisms by which TFAs actually work at the cellular level. It is unknown what levels of TFAs are clinically significant and it is not clear how TFAs are associated with cardiac arrhythmias or sudden cardiac arrest. It was hypothesized that TFAs affect membrane structure, thus altering enzymatic pathways that may subsequently induce cardiac arrhythmias and sudden death. Moreover observational studies by Mozaffarian et al. (2009) showed that a higher CHD risk is related to TFA from industrial sources. Because ruminant fat contains low levels of TFA (<6% of fatty acids), the amounts of ruminant TFA consumed are low in most countries studied (generally <1 energy%). Thus, even when the total ruminant fat intake is relatively high, the potential amount of TFA from this source is still quite modest. In the amounts actually consumed, ruminant TFA is not a contributor to CHD risk.

Breast cancer There is conflicting evidence concerning the possible role of TFA in breast cancer. Kohlmeier et al. (1997) investigated the relationship between TFA and postmenopausal breast cancer in European populations differing greatly in their dietary fat intakes. The adipose concentration of TFA showed a positive association with breast cancer, not attributable to differences in age, body mass index, exogenous hormone use, or socio-economic status. These findings suggested an association of adipose stores of TFA with postmenopausal breast cancer in European women, but require confirmation in other populations, with concomitant consideration of the potential roles of dietary saturated and monounsaturated fats.

The analysis of trans and cis fatty acids levels in blood serum of women showed that breast cancer risk increased with the increase in trans fatty acid level, reflecting processed food consumption. It was reported that women with elevated serum levels of trans fatty acid have double the risk of developing breast cancer as compared to women with the lower levels.

Pregnancy Based on results from animal studies, it was previously assumed that trans fatty acids do not cross the placenta, and that the foetus is therefore protected against trans fatty acids (Stender et al. 1994). However more recent studies on humans have shown that trans fatty acids are transferred to the foetus, as they were found in the same levels in the blood of newborn infants as in that of mothers (Berghaus et al. 1998; Elias and Innis 2001). In animal experiments, a high intake of industrially produced trans fatty acids inhibits the formation of long-chain polyunsaturated fatty acids (LCPUFAs) from their precursors (Pax et al. 1992). Theoretically, something similar may apply in humans (Koletzko 1992). LCPUFAs are important for both growth and the development of vision and the central nervous system in foetus. Elias and Innis (2001) showed that trans fatty acid levels including conjugated linoleic acid (CLA) in the umbilical blood of neonates reflected the mother's levels of trans fatty acids in the blood and thus the mother's trans fatty acid intake. The pregnancy period was also found to be shorter in mothers with higher trans fatty acid level in the infant's blood. n-3 fatty acids from fish oils prolonged pregnancy (Olsen et al. 1992) while trans fatty acids appear to shorten it. n-3 fatty acids inhibit the contraction in uterine cells by virtue of an effect on the ion channels of these cells, thus prolonging pregnancy. Trans fatty acids may have the opposite effect. Mother's intake of trans fatty acids is negatively associated with levels of polyunsaturated fatty acids in the blood of newborn infants, it is advisable to minimise the intake of trans fatty acids during pregnancy (Hornstra 2000). Another surprising observation was the finding of an association between a high intake of trans fatty acids and the risk of preeclampsia (pregnancy induced hypertension) (Yli-Jama et al. 2002). In this study, trans fatty acid intake was estimated by the trans fatty acid content of the cell walls of red blood cells. It was noticed that women who developed preeclampsia had approximately 30% higher trans fatty acid levels in red blood cells than women who did not develop this disorder.

Interference with essential fatty acids Essential fatty acids (EFA) are transformed in the body by a series of reactions into long chain polyunsaturated fatty acids essential for

development of the nervous system and eyesight (Sugano and Ikeda 1996). TFA compete with EFA for the enzyme systems involved in these reactions. High intakes of TFA have been shown to influence the metabolism of EFA in experimental animals when the EFA intake was low. Deficiency of EFA is found only in abnormal circumstances in human adults. However, new-born infants, and especially if premature, show borderline deficiency in EFA, and their TFA intake from the mother's milk is related to her TFA intake.

Colon cancer Slattery et al. (2001) reported that the men and women above age of 67 years those did not use non steroidal anti-inflammatory drugs (NSAIDs) were at a 50% greater risk of developing colon cancer when they consumed high levels of trans-fatty acids. Women who were estrogen negative i.e. not taking hormone replace therapy after menopause, had a twofold increase in risk from high levels of trans-fatty acids in the diet, while women who were estrogen positive did not experience an increased risk of colon cancer, regardless of level of trans-fatty acids consumed. It has been hypothesized that trans-fatty acids could increase risk of cancer through alteration of immune response, cell wall integrity, and prostaglandin synthesis.

Diabetes Analysis of the Nurses' Health Study after 14 years observation showed that the risk of the development of type-II diabetes was associated with trans fatty acid intake (Salmeron et al. 2001). It was observed that as the intake of industrially produced trans fatty acids in the USA is on average 3% energy, a reduction in trans fatty acid intake of 2% energy could reduce the incidence of type-II diabetes by 40% if the fats containing the trans fatty acids were consumed in their original unhydrogenated form. It was not possible, however, to find such an association in either the Iowa Women Study (Meyer et al. 2001) or the Health Professional's Study (Wahle and James 1993). Studies carried out at National Institute of Nutrition (NIN), Hyderbad, India to evaluate the effects of TFA from vanaspati in rats showed that both saturated fatty acids (SFA) (5 g/100 g,10% energy) and TFA (3% energy) increased insulin resistance (decreased insulin sensitivity). However, the effects of TFA were greater than SFA in increasing insulin resistance. Increasing dietary linoleic acid did not prevented TFA induced increase in insulin resistance; it becomes necessary to reduce the absolute intake of TFA (Ghafoorunissa 2008).

Obesity Research indicates that trans fat may increase weight gain and abdominal fat deposits, despite a similar caloric intake. Industrially produced trans fatty acids and trans fatty acids from ruminants contain calories in the same quantities as other edible fats. A Swedish study indicates that certain conjugated linoleic acid isomers that are present only in very low levels in ruminant fat increase the insulin resistance of men with abdominal obesity (Ricerus et al. 2002). A six year study revealed that monkeys fed on a trans fat diet gained 7.2% of their body weight, as compared to 1.8% for monkeys on a monounsaturated fat diet (Kavanagh et al. 2007).

Allergy The incidence of asthma, allergic cold and asthmatic eczema in children aged 13–14 years was investigated in selected centres around the world (Weiland et al. 1999). A positive association was found between the intake of trans fatty acids and these diseases. Such an association was not observed for the intake of monounsaturated and polyunsaturated fatty acids (Willett et al. 1993).

Alternative approach to trans fats

Increased consumer awareness of the health implications of TFAs, has resulted in local and state efforts to limit or ban their use by restaurants and foodservice establishments. Food manufacturers are using or developing basically four technological options to reduce or eliminate TFA in their products. These options include:

Modification of the hydrogenation process Hydrogenation i.e. saturating some double bonds and converting others to the trans configuration is a common technique to provide firmness and plasticity to shortenings, thus, enabling the production of solid and semi solid fats. Modifying the conditions of hydrogenation (e.g. pressure, temperature, and catalyst) affects the FA composition of the resulting oil, including the amount of TFA formed, and properties such as melting point and solid fat content of the oil. It is possible to make equivalently performing low-trans fats by increasing the degree of hydrogenation, which reduces the level of TFA but increases the level of saturated fatty acids. Modification of the hydrogenation process can be used to prepare low-trans baking shortenings. Low or zero-trans baking fats may have increased levels of stearic acid from the hydrogenation of α-linolenic, linoleic, and oleic acids, and also significant levels of palmitic acid for functionality.

Use of interesterification The interesterification process rearranges the distribution of the fatty acids either chemically or enzymatically within and between the triglycerides thus the fatty acid distribution is altered but the fatty acid composition remains same. Interesterification modifies the melting and crystallization behaviour of the fat, thus producing fats with the desirable physical properties of trans fats but without TFA. One current application of this process is in the production of trans-free or low-trans fats spreads, margarine, and shortening. Several human studies

have shown no significant effects of interesterified fats on blood lipid parameters (Hunter 2001; Meijer and Weststrate 1997; Nestel et al. 1995).

Use of fractions high in solids from natural oils Fractions high in solids derived from natural oils, namely coconut, palm, and palm kernel oils, are not new to the food industry and have been components of functional ingredients for years. If fat is melted and cooled slowly to below its melting point, the triglycerides with a higher melting point than the tempering temperature will eventually form crystalline material, which can be relatively easily centrifuged or filtered off from the liquid part. Many commercially available fractions come from palm and palm kernel oils. They can be used successfully either as single fractions or in combination with other fractions to meet specific needs.

Use of trait-enhanced oils Trait-enhanced oils generally fall into three categories: high-oleic acid oils, such as high-oleic sunflower and canola oils, mid-range oleic acid oils, such as mid-oleic sunflower and soybean oils, and low-linolenic acid oils, such as low-linolenic canola and soybean oils. (The term "low linolenic" commonly refers to oil containing about 1–3% α-linolenic acid. Soybean oil typically contains about 7%, and canola oil, about 10% α-linolenic acid.) These types of oils are derived through traditional plant breeding or biotechnological methods. All of these trait-enhanced oils have good oxidative stability making them suitable for frying, spraying, and some bakery applications. These modification techniques offer the chance to minimise and control the trans content of oil blends, and can be used to successfully formulate trans-free hardstocks. However, the combination of these techniques, leads to a greater variety of hardstocks with a wider range of physical properties such as solid fat phase and melting point behaviour.

Recent trends have indicated that many frying fats in the fast-food industry have been replaced by medium- and high-stability vegetable oils, resulting in a virtual elimination of trans fats in products fried in these fats and a significant reduction of saturated fats as well (usually by more than 50%). The possible evolution of these changes is shown in Table 3 (Skeaff 2009).

Analysis of trans fatty acids in food products

There are two official methods for the quantification of trans fatty acid accepted by the American Oil Chemists' Society (AOCS) and the Association of Official Analytical Chemists (AOAC) namely, capillary gas chromatography (GC) and Fourier transform infrared (FTIR) spectroscopy in conjunction with the attenuated total reflectance (ATR) cell. The technique of Fourier transform infrared (FTIR) spectroscopy is capable of the determination of isolated trans-double bonds in commercial fat and oils samples with greater ease and accuracy, since it is no longer necessary to derivatize or to dissolve in solvents prior to the analysis; automation of sample handling and data collection is also possible.

Labelling of trans fat

The Food and Drug Administration (FDA) requires that the Nutrition Facts panel list the amount of trans fat in a serving of food if a serving contains 0.5 g or more of trans fatty acids, this is listed on the line below the listing of saturated fat. For nutrition labeling purposes, trans fats are defined as the sum of all unsaturated fatty acids that contain one or more isolated, non conjugated, double bonds in a trans geometric configuration. Conjugated fatty acids with a trans double bond, including conjugated linoleic acid (commonly known as CLA) isomers, are excluded from this definition of trans fats. There is no Daily Value for trans fat. Instead, the Institute of Medicine recommends we keep our intake of trans fats to as near zero as possible. The World Health Organization (WHO) recommended that governments around the world phase out partially hydrogenated oils if trans-fat labelling alone does not spur significant reductions.

Trans fats in Indian food

Saturated fats were supposed to be the main cause of heart disease over years, but now from various studies it has been observed that trans fats are the main culprits. In India, trans fats are consumed a lot in the form of vanaspati i.e. hydrogenated vegetable oil. Vanaspati is the cheaper source

Table 3 Evolution of deep-frying fats

Time	Type of fat used	Trans	Saturated
Traditional practice	Partially hydrogenated oils	30%–40%	20%
Present	Blends of palm olein, cottonseed, sunflower seed, canola, and so on	Zero	30%–40%
Future	High-oleic forms of sunflower, soybean, canola, and so on	Zero	<20%

Skeaff 2009

of fat and improves taste as well. WHO has recommended that TFA intake as a% of energy should not exceed 1%. The total fat intake as a% of energy should not be less than 15% and should not exceed 30%. The intake of Saturated Fat (SFA) as a% of energy should not exceed 10% (7% for cardiac patients). Food preparations enjoyed frequently by Indian people are prepared in vanaspati thus contributing trans fat in diet (Table 4). Trans fat is also present in sweets, chocolates, spreads, soups, salad dressings and snacks. In rural and urban India the fat consumption is around 20 and 30 g/day, respectively, according to diet studies (National Consumption Survey data by NIN 2009) (FSSA 2010). If 10% TFA is permitted in vanaspati, a person consuming 2,000 Kcal derived from food which contains 20 and 30 g vanaspati/day will derive 0.9 and 1.35% energy from the TFA. (This shows that even at 10% TFA level there is health risk at 30 g of vanaspati consumption per day (which exceeds the 1% energy, which is the limit for TFA recommended by WHO). The trend of eating out in the urban population and consumption of food in hotels and restaurants add to criticality as the food prepared there is very high in trans fats. According to the latest recommendations, trans fat in oil should not exceed 2% of the total fat. However, the laboratory tests conducted by Delhi based Centre for Science and Environment (CSE) found trans fat levels to be as high as 23% in some vanaspati brands liberally consumed in India. Trans fats levels were, however, lower in *desi* ghee, butter and the refined oils. The World Health Organization has predicted that deaths due to circulatory system diseases will double between 1985 and 2015 in India.

To regulate the TFAs in partially hydrogenated vegetable oils, the issue was considered in the Third meeting of the Food Authority held on 26th November, 2009 where it was recommended to fix a limit of not more than 10% trans-fatty acids in partially hydrogenated vegetable oils. It was also recommended that a national consultation may also be organized to obtain feedback from consumers and industry and the scientific community for implementation of the regulation. Hence, National Institute of Nutrition, Hyderabad conducted a national consultation by inviting participants representing all stakeholders on 29.01.2010. Considering the

Table 4 Trans fat in some commonly consumed Indian foods (g/100 g)

Some Indian food	Energy (calories)	Fat (gm)	Total trans fat (gm)	TFA as fat%	TFA en%
Common Indian sweets					
Barfi (Heat dried milk, ghee and sugar)	409.0	19.7	8.4	42.5	18.4
Sweet biscuits	349.0	10.2	4.8	47.1	12.4
Butter biscuits	482.0	17.3	0.0	0.0	0.0
Plain cake	492.0	27.7	0.0	0.0	0.0
Pinni (roasted flour, heat dried milk, sugar and ghee)	492.0	17.4	0.3	1.9	0.6
Gulab-jamun (deep-fried heat dried milk in sugar syrup)	387.0	11.4	6.1	53.0	14.1
Halwa (fried flour, sugar syrup, ghee and nuts)	263.0	12.3	6.3	51.3	21.6
Jalebi (fried fermented flour, in sugar syrup)	494.0	34.8	17.7	50.8	32.2
Churi (roasted flour mashed with ghee, powered sugar and nuts)	454.0	22.6	0.4	1.9	0.9
Kheer (creamy rice pudding)	141.0	7.7	0.0	0.0	0.0
Shakarpara (fried flour with shortening, dipped in sugar syrup)	403.0	6.9	3.1	45.4	7.0
Indian snacks/savories					
Indian bread (leavened baked flour)	275.0	5.0	1.9	38.0	6.2
Potato kachori (deep-fried pastry filled with potatoes)	603.0	10.5	5.6	53.0	30.3
Chewra (deep-fried flaked rice, sugar and nuts)	420.0	25.9	10.6	41.0	22.7
Paapri (deep-fried white flour with shortening)	444.0	19.5	10.2	52.2	20.6
Plain khichri (steamed cooked rice pulse and ghee)	168	7.4	4.0	54.1	21.4
Vegetable biryani (rice, ghee, meat, fish or vegetable)	148.0	6.0	3.1	51.7	18.9
Mathri (fried flour with shortenings, rolled flat)	495.0	30.8	16.3	53.0	29.7
Samosa (a triangular deep-fried pastry containing vegetable or meat)	256.0	13.0	3.3	25.4	11.6
Potato puri (deep-fried unleavened wheat bread filled with potato)	247.0	9.5	4.8	50.9	17.6

Aggarwal et al. 2008

recommendations of the national consultation meet FSSAI has proposed the following the following with regard to TFA limits:-

- The TFA level in PHVO should be below 10% and brought down to 5% in 3 years. A phasing in period may be given to industry after the date of notification.
- Existing melting point regulation which is 31°–41 °C for partially hydrogenated vegetable oils, bakery shortening and margarines, interesterified vegetable fat and other fats made using vegetable oils be raised only to the extent that would facilitate bringing down the TFA level to the above limits.
- There is need to look into the feasibility for laying down the limits of Saturated Fatty Acids (SFA) in vanaspati and other fats. This is being thought of because if the melting point is raised, it will lead to increase in saturation of partially hydrogenated vegetable oils. WHO had recommended that not more than 1% and not more than 10% of energy in diet be derived from TFAs and SFAs, respectively.
- Palm Stearin content may be permitted only in interesterified fat and not approved for blending of oils or to be used as such.
- There should be mandatory labelling of TFA & SFA content on vanaspati packs, edible oils or any other product containing TFA from vanaspati sources.
- Enzymatic esterification for production of vanaspati for regulating trans fatty acids can be considered. But it being a costly alternative, may take time for implementation.
- There is currently a limit on blending of more than two oils and a minimum requirement of 20% for each oil used for blending. These limits have been imposed to facilitate detection of adulteration. There is a demand for reviewing this restriction to facilitate greater use of other oils by industry and facilitate balance in SFA : MUFA : PUFA components.

Conclusions

Trans fatty acids have several beneficial aspects for processed foods owing to their characteristic structures. These very characteristic structures, in turn, have been suspected to be associated with the possibility that trans fatty acids affect the development of several health problems, including coronary heart disease, and foetal and infant neurodevelopment and growth, and childhood allergies etc. There is considerable interest in zero- and low-trans fats among food manufacturers, and current use of such products is increasing. But banning all TFA from the diet would be detrimental as this would include banning trans fats that could be positive for health, such as vaccenic acid. Ruminant animal products, such as meat and dairy are rich in essential nutrients, such as protein, calcium, and iron, which are difficult to obtain from plants or other sources. To ban these foods would have detrimental effects on the population at large, with the most potentially serious ramifications for infants, who require a variety of fatty acids for growth and development. Four independent strategies are needed to limit trans fat intake i.e. health care providers should advise their patients about how to minimize the intake of trans fats. Consumers should learn to recognize and avoid products containing trans fats. Restaurants and food manufacturers should use alternative fats in food production and preparation and local, state, and national government agencies should aid these efforts by enforcing legislation that limit trans fat use. These steps should help reduce the consumption of trans fatty acids, likely resulting in substantial health benefits. In addition, development of new TFA free products requires more research to determine the health-related effects, as it would be impractical to replace TFA with products that may be just as detrimental, or even worse.

References

Aggarwal A, Gupta R, Varma K, Mathur B (2008) High trans fatty acid content in common Indian fast foods. Nutr Food Sci 38(6):564–569

Aro A, Jauhiainen M, Partanen R, Salminen I, Mutanen M (1997) Stearic acid, trans fatty acids, and dairy fat: effects on serum and lipoprotein lipids, apolipoproteins, lipoprotein (a), and lipid transfer proteins in health subjects. Am J Clin Nutr 65:1419–1426

Ascherio A, Hennekens CH, Buring JE, Master C, Stampfer MJ, Willett WC (1994) Trans fatty acid intake and risk of myocardial infarction. Circulation 89:94–101

Berghaus TM, Demmelmair H, Koletzko B (1998) Fatty acid composition of lipid classes in maternal and cord Plasma at birth. Eur J Pediatr 157:763–768

Caggiula AW, Mustad VA (1997) Effects of dietary fat and fatty acids on coronary artery disease risk and total and lipoprotein cholesterol concentrations: epidemiologic studies. Am J Clin Nutr 65:1597S–1610S

Elias SL, Innis SM (2001) Infant plasma trans, n-6, and n-3 fatty acids and conjugated linoleic acids are related to maternal plasma fatty acids, length of gestation, and birth weight and length. Am J Clin Nutr 73:807–814

FSSA (2010) Food Safety and Standards Authority of India Proposes Regulation of Trans Fatty Acids (TFAs) in Partially Hydrogenated Vegetable Oils (PHVOs) http://www.fssai.gov.in/Website/LinkClick.aspx?fileticket=SvU1nfLZRrc%3D&tabid=94. Accessed 10 November 2010

Ghafoorunissa G (2008) Role of trans fatty acids in health and challenges to their reduction in Indian foods. Asia Pac J Clin Nutr 17:212–215

Gould AL, Rossouw JE, Santanello NC, Heyse FJ, Furberg CD (1998) Cholesterol reduction yields clinical benefit: impact of stain trials. Circulation 97:946–952

Hornstra G (2000) Essential fatty acids in mothers and their neonates. Am J Clin Nutr 71:1262S–1269S

Hunter JE (2001) Studies on effects of dietary fatty acids as related to their position on triglycerides. Lipids 36:655–668

Judd JT, Clevidence BA, Muesing RA, Wittes J, Sunkin ME, Podczasy JJ (1994) Dietary trans fatty acids: effects of plasma lipids and lipoproteins on healthy men and women. Am J Clin Nutr 59:861–868

Kavanagh K, Jones KL, Sawyer J, Kelley K, Carr JJ, Wagner JD, Rudel LL (2007) Trans fat diet induces abdominal obesity and changes in insulin sensitivity in monkeys. Obes Res 15:1675–1684

Keys A (1980) Coronary heart disease, serum cholesterol, and the diet. Acta Med Scand 207:153–160

Kohlmeier L, Simonsen N, Van't Veer P et al (1997) Adipose tissue trans fatty acids and breast cancer in the European community multicenter study on antioxidants, myocardial infarction, and breast cancer. Cancer Epidemiol Biomark Prev 6(9):705–710

Koletzko B (1992) Trans fatty acids may impair biosynthesis of long-chain polyunsaturates and growth in man. Acta Paediatr 81:302–306

Kromhout D, Lezenne CC (1984) Diet, prevalence and 10-year mortality from coronary heart disease in 871 middle-aged men Zutphen Study. Am J Epidemiol 119:733–741

Lichtenstein AH, Ausman LM, Jalbert SM, Schaefer EJ (1999) Effects of different forms of dietary hydrogenated fats on serum lipoprotein cholesterol levels. N Engl J Med 340:1933–1940

Liu WH, Stephen Inbaraj B, Chen BH (2007) Analysis and formation of trans fatty acids in hydrogenated soyabean oil during heating. Food Chem 104(4):1740–1749

Meijer GW, Weststrate JA (1997) Interesterification of fats in margarine: effect on blood lipids, blood enzymes, and hemostasis parameters. Eur J Clin Nutr 51:527–534

Mensink RP (2005) Metabolic and health effects of isomeric fatty acids. Curr Opin Lipidol 16:27–30

Mensink RP, Katan MB (1990) Effect of dietary trans fatty acids on high-density and low density lipoprotein cholesterol levels in healthy subjects. N Engl J Med 323:439–445

Meyer KA, Kushi LH, Jacobs DR, Folsom AR (2001) Dietary fat and incidence of type II diabetes in older Iowa women. Diabetes Care 24:1528–1535

Mozaffarian D, Katan MB, Ascherio A, Stampfer MJ, Willett WC (2006) Trans fatty acids and cardiovascular disease. N Engl J Med 354:1601–1613

Mozaffarian D, Aro A, Willett WC (2009) Health effects of trans-fatty acids: experimental and observational evidence. Eur J Clin Nutr 63(2):S5–S21

Nestel PJ, Noakes M, Belling GB, McArthur R, Clifton PM (1995) Effect on plasma lipids of interesterifying a mix of edible oils. Am J Clin Nutr 62:950–955

Olsen SF, Sørensen JD, Secher NJ, Hedegaard M, Henriksen TB, Hansen HS, Grant A (1992) Randomised controlled trial of effect of fish-oil supplementation on pregnancy duration. Lancet 339:1003–1007

Pax J, Douglass L, Sampugna J (1992) Effects of linolenic and trans-fattyadics on neonatal survival of C57BL/6 Mice. J Nutr Biochem 3(7):342–348

Ricerus U, Arner P, Brismar K, Vessby B (2002) Treatment with dietary trans10- cis12 conjugated linoleic acid causes isomer-specific insulin resistance in obese men with the metabolic syndrome. Diabetes Care 25:1516–1521

Salmeron J, Hu FB, Manson JE, Stampfer MJ, Colditz GA, Rimm EB, Willett WC (2001) Dietary fat intake and risk of type II diabetes in women. Am J Clin Nutr 73:1019–1026

Skeaff CM (2009) Feasibility of recommending certain replacement or alternative fats. Eur J Clin Nutr 63:S34–S49

Slattery ML, Benson J, Ma KN, Schaffer D, Potter JD (2001) Trans-fatty acids and colon cancer. Nutr Cancer 39(2):170–175

Stender S, Dyerberg J, Hølmer G, Ovesen L, Sandström B (1994) Transfedtsyrers betydning for sundheden. A report from the Danish Nutrition Council. Publ No. 2. Copenhagen 1994

Sugano M, Ikeda I (1996) Metabolic interactions between essential and trans-fatty acids. Curr Opin Lipidol 7:38–42

Tsuzuki W (2010) Cis–trans isomerization of carbon double bonds in monounsaturated triacylglycerols via generation of free radicals. Chem Phys Lipids 163:741–745

Wahle KWJ, James WPT (1993) Isomeric fatty acids and human health. Eur J Clin Nutr 47:828–839

Weggemans RM, Rudrum M, Trautwein EA (2004) Intake of ruminant versus industrial trans fatty acids and risk of coronary heart disease—what is the evidence? Eur J Lipid Sci Technol 106:390–397

Weiland SK, von Mutius E, Hüsing A, Asher MI (1999) On behalf of the ISAAC Steering Committee. Intake of trans fatty acids and prevalence of childhood asthma and allergies in Europe. Lancet 353:2040–2041

Willett WC, Stampfer MJ, Manson JE, Colditz GA, Speizer FE, Rosner BA, Sampson LA, Hennekens CH (1993) Intake of trans fatty acids and risk of coronary heart disease among women. Lancet 341:581–585

Williams MA, King IB, Sorensen TK, Zingheim RW, Troyer BL, Zebelman AM, Luthy DA (1998) Risk of preeclampsia in relation to elaidic acid (Trans Fatty Acid) in maternal erythrocytes. Gynecol Obstet Investig 46:84–87

Yli-Jama P, Meyer HE, Ringstad J, Pedersen JI (2002) Serum free fatty acid pattern and risk of myocardial infarction: a case-control study. J Intern Med 251:19–28

Zock PL, Vries JHM, Fouw NJ, Katan MB (1995) Positional distribution of fatty acids in dietary triglycerides: effects on fasting blood lipoprotein concentrations in humans. Am J Clin Nutr 61:48–55