STUDIES ON DEVELOPMENT OF *ALOE VERA* FORTIFIED LOW CALORIE FUNCTIONAL APPLE SPREAD

Thesis

by

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(H-2018-35-M)

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CERTIFICATE – I

This is to certify that the thesis titled **"Studies on development of *Aloe vera* fortified low calorie functional apple spread"** submitted in partial fulfilment of the requirements for the award of the degree of **MASTER OF SCIENCE FOOD TECHNOLOGY** in the discipline of **Food Science and Technology** to Dr. Yashwant Singh Parmar University of Horticulture & Forestry, (Nauni) Solan (HP)-173 230 is a record of bonafide research work carried out by **Ms Tamanna Sharma (H-2018-35-M)** daughter of Shri Dharam Pal under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been fully acknowledged.

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Major Advisor

CERTIFICATE - II

This is to certify that the thesis titled, **"Studies on development of *Aloe vera* fortified low caloric functional apple spread"** submitted by **Ms Tamanna Sharma (H-2018-35-M)** daughter of Shri Dharam Pal to Dr. Yashwant Singh Parmar University of Horticulture & Forestry, (Nauni) Solan (HP)-173 230 India in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE FOOD TECHNOLOGY** in the discipline of **Food Science and Technology** has been approved by the Student's Advisory Committee after an oral examination of the student in collaboration with the Internal Examiner.

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CONTENTS

ABBREVIATIONS USED

%	:	Per cent
@	:	At the rate
3-D	:	3 dimensional
ADI	:	Acceptable daily intake
ANOVA	:	Analysis of variance
cc	:	Cubic centimeter
cfu	:	Colony forming unit
cm	:	Centimeter
DPPH	:	Diphenyl picryl hydrazyl
et al.	:	Co-workers
FAO	:	Food and Agriculture Organization
FOS	:	Fructo-oligosaccharide
FDA	:	Food and Drug Administration
FSSAI	:	Food Safety and Standards Authority of India
g	:	Gram
GAE	:	Gallic Acid Equivalent
GRAS	:	Generally Recognized as Safe
i.e.	:	That is
IASC	:	International Aloe Science Council
Kcal	:	Kilocalories
kg	:	Kilo gram
KMS	:	Potassium Metabisulphite
LCSs	:	Low calorie sweeteners
mg	:	Milligram
min	:	Minute
mL	:	Millilitre
mm	:	Millimeter
mol	:	Mole
MPa	:	Mega pascal
NEB	:	Non-enzymatic browning

NHB	:	National Horticulture Board
nm	:	Nanometer
NNS	:	Non-nutritive sweeteners
ºB	:	Degree brix
ºC	:	Degree celcius
OD	:	Optical Density
ppm	:	Parts Per Million
R & D	:	Research and Development
RPM	:	Revolutions Per Minute
Rs	:	Rupees
RSM	:	Response Surface Methodology
RTS	:	Ready-To-Serve
SE	:	Standard Error
sec	:	Seconds
TLC	:	Thin Layer Chromatography
TPC	:	Total plate Count
TSS	:	Total Soluble Solids
UV-vis	:	Ultravoilet-visible
var	:	variety
WHO	:	World Health Organization

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Chapter-1

INTRODUCTION

The modern lifestyle characterized as sedentary activities with high calorie consumption and lack of physical activities has been reported to cause many health problems such as obesity, diabetes, hypertension etc. (Sharma *et al.,* 2019). As a result, the present consumers are now more concerned about their health and desired foods which offer less fat/cholesterol/sugar and fewer calories in addition to convenience, good quality, safety and taste at an economical price (Khan and Aroulmoji, 2018; Sutwal *et al.,* 2019). Such food products are a category of *functional food products,* which not only satisfy hunger and provide necessary nutrients, but also support prevention of nutrition related diseases (Sharma *et al.*, 2020). Fruits and vegetables are considered as protective foods as they contain numerous phytochemical or bioactive compounds like polyphenols, carotenoids, vitamins, minerals etc. which provide protection against many diseases (Wargovich, 2000; Sharma *et al.,* 2012). Several epidemiological studies have been reported that daily consumption of such products can reduce the risk of premature ageing, strokes, stress, cancer and heart diseases primarily due to the synergistic action of various bioactive compounds (Bhardwaj and Pandey, 2011; Sharma *et al*., 2018).

Apple (*Malus* × *domestica* Borkh) is a pomaceous fruit and belongs to family Rosaceae. In India, the annual production of apple was 2,371 thousand metric tonnes from an area of 307 thousand hectare (NHB, 2018). However, the annual production of apple in Himachal Pradesh is 4,46,570 metric tonnes from an area of 1,12,630 hectare (NHB, 2018). It is one of the most frequently consumed fruits in many parts of the world because it is a rich source of phytochemicals like quercetin, catechin, phloridzin and chlorogenic acid besides vitamins (A and C), carbohydrates, minerals and dietary fibers. They also possess pharmacological activities such as inhibit cancer cell proliferation, decreases lipid oxidation, very strong antioxidant activity, lowers the cholesterol, prevents constipation and helps to control obesity (Boyer and Liu, 2004). Apple processing industry is one of the major industries of the state and about 20 per cent of its production is being processed into different value added products like juices, concentrates, wine, cider, canned slices etc. (Joshi *et al.,* 1991; Joshi, 1997; Kaushal *et al.,* 2002; Gulhane *et al.,* 2015). However, fruit spread is another product gaining demand in the market and hence efforts can be made to prepare apple spread.

On the other hand, *Aloe vera* (syn. *Aloe barbadensis* Miller*)* belonging to the family Asphodelaceae (Liliaceae) is one of the most important herbal plant gifted by nature and its juice is called *nature's tonic*. It has varied functional properties like analgesic, anti-inflammatory, wound healing, immune modulating and anti-tumor activities as well as antiviral, anti-bacterial and antifungal properties (Sharma *et al.*, 2018). In addition, the oral ingestion of *Aloe vera* gel has been recommended for facilitating digestion, aiding blood and lymphatic circulation, besides improving kidney, liver and gall bladder functions (Eshun and He, 2004). However, poor appearance and bitter taste of *Aloe vera* gel/juice are some of the consumer disliking factors and major problems to the food industries not to use it in the products (Sharma *et al*., 2015; Mishra and Sangma, 2017). Therefore, it becomes obligatory to standardize a technology to develop palatable *Aloe vera* based functional food products. Nowadays, it is being utilized as a potential ingredient in a wide range of health foods and drinks by developing new formulations of the products (Ramachandra and Rao, 2008; Ahlawat and Khatkar, 2011; Ramachandran and Nagarajan, 2014; Sharma *et al*., 2015). But, very little scientific work is available on *Aloe vera* based spread and like products. Thus, utilization of *Aloe vera* for the preparation of functional fruit spread can be attempted in order to develop a innovative product.

Fruit spreads are jam like products which are having smooth texture and uniform spreadability. They are characterized by high fruit and reduced sugar content compared to jams (Holzwarth *et al.,* 2013). Furthermore, jams or like products commercially available in the market are generally concentrated source of sugar and according to FSSAI-2006 specifications, these should contain at least 65 per cent of total soluble solids (TSS). Although, sugar play an important role in providing flavour, appearance, colour, taste and texture to the finished products (Aggarwal *et al.,* 2016), but such products give quick burst of calories and excess calorie intake has been reported to be partially responsible for various health diseases like hypertension, obesity, diabetes and overweight (Sharma and Joshi, 2015; Sutwal *et al.*, 2019). Whereas, modern consumers are now more concerned about the caloric value of food they consume and wish to enjoy the pleasure of sweetness without extra calories (Alkhatib *et al.,* 2017; Sutwal *et al.,* 2019). Therefore, use of low calorie sweeteners for replacement of sugar (sucrose) seems to be an effective approach. Low calorie sweeteners are those additives which have lesser glycemic index and provide less than 2 per cent of calorie value of sucrose per equivalent unit of sweetening capacity (Pandey and Nigam, 1987). Several low-calorie sweeteners (LCSs) have been used by researchers in many foods and beverages, for reducing total calories, while maintaining their palatability (Barwal *et al.*, 2002; Wiebe *et al.,* 2011; Abolila *et al.,* 2015; Sharma and Tandon, 2015; Sharma *et al.*, 2018). Sweetos (FOS-sucralose) is one such sweetener which is a liquid bulk sweetener and impart sweetness equivalent to sugar because it contains combination of fructooligosaccharide (FOS) and sucralose (Aggarwal *et al.* 2016; Sharma *et al.,* 2020). Sucralose is a non-nutritive sweetener, which is safe, poorly absorbed, non-caloric, non-cariogenic and has no effect on blood glucose or insulin levels of the consumers (Binns, 2003). Whereas, fructooligosaccharides (FOS) are short chain of fructose molecules which are excellent source of soluble fiber and do not add calorie because they are non digestible by stomach enzymes (Lorenzoni *et al.*, 2015). The FOSs have attracted special attention because of their prebiotic properties, reduced calorie value and also their sweet taste being very similar of sucrose (Ghavidel *et al.*, 2014; Sharma *et al.*, 2020).

Keeping above facts in view, the present research work was conducted to explore the possibility of developing *Aloe vera* fortified low calorie functional apple spread for the benefit of masses in general and health conscious peoples in particular with following objectives:

i) To optimize different process parameters for the development of *Aloe vera* fortified apple spread

ii) To evaluate the suitability of sweetos for the development of *Aloe vera* fortified low calorie functional apple spread

iii) To evaluate the product for physico-chemical, nutritional, sensory and microbiological quality during storage

Chapter-2

REVIEW OF LITERATURE

Now-a-days, nutrition is a vital factor in foods, not only in the growth but also in the prevention and treatment of diseases. Nutrition is also fundamental for the maintenance of good health and functionality (Ohlhorst *et al.,* 2013). The concept of "*Functional Foods*" was given by Japanese Ministry of Health and Welfare, to instigate a regulatory system for foods possessing health benefits with the aim to improve the overall health of country's population and to keep a check on the increasing healthcare costs (Kaur and Singh, 2017). A food can be regarded as functional, if it has beneficial effects on target functions in the body besides nutritional effects that is relevant to health and well being. Whole foods such as fruits, vegetables and medicinal herbs represent the simplest form of functional food due to their richness in biologically active compounds (Hardy, 2000; Day *et al.,* 2009; Sharma *et al.,* 2012; Kapoor and Ranote, 2015). Various epidemiologic studies over decades have indicated that a continuous consumption of fruits and vegetables in regular diet is often correlated with decreased risk of cancer, cardiovascular diseases and age-related macular degeneration (Sharma *et al.,* 2019). Whereas, functional compounds when incorporated in a basic food material are expected to perform certain functions and thus make the food functional food (Sharma, 2015). Among fruits, apple is one of the most important temperate fruits having multiple health benefits due to its nutritional and functional properties. It is a good source of vitamin C, potassium and fiber. It contains about 11 per cent sugars, 0.3 per cent proteins, 14 per cent carbohydrates, 4 per cent vitamins and minerals and remaining part of apple contains water (Boyer and Liu, 2004). The high intake of apple has been reported to reduce the risk of development of colorectal adenomas (Michels *et al.,* 2006). Apples are protective against cardiovascular diseases due to cholesterol lowering ability, whereas, the polyphenols which are present in it have health promoting antioxidant properties (Sluis *et al.,* 2002).

Aloe vera is one of the oldest known medicinal plants gifted by nature. The *Aloe vera* leaf contains high water content, ranging from 99 per cent to 99.5 per cent, while the remaining 0.5–1.0 per cent solid material is reported to contain over 200 different potentially active compounds, including anthraquinones, anthrones and their glycosides chromones, carbohydrates, proteins, glycoproteins, vitamins, minerals, enzymes, simple and complex polysaccharides, phenolic compounds and organic acids (Rodriguez *et al*., 2010; Boudreau *et* *al*., 2013; Manvitha and Bidya, 2014). Commercially, *Aloe vera* is being used in pills, sprays, ointments, lotions, liquids, drinks, jellies, and creams. Though, its juice/pulp is being used in the development of several functional food products, but very little scientific work is available on jams and spreads (Manvitha and Bidya, 2014). Further, the fruit jams or like product contains high amount of table sugar which provide quick burst of energy and a large amount of calories. Unfortunately, high calorie intake has been reported partially responsible for hypertension, diabetes, cardiovascular diseases and obesity. Whereas, low calorie sweeteners can provide consumers a way to enjoy the sweet taste of fruit jams or like products, besides reduced calorie intake and without compromising nutritional value of the product.

Thus, fortification of apple jam or like products with *Aloe vera* and replacement of sucrose low calorie sweetener can be attempted to make functional low calorie jam/spread. Some of the scientific research work done in India and abroad on various aspects of *Aloe vera*, incorporation of pulp, preparation of functional products, non-nutritive sweeteners, storage studies etc. has been reviewed under the following heads:

2.1 Present status of apple and *Aloe vera* in India
2.2 Physico-chemical composition, functional and nutraceutical properties of apple and *Aloe vera*
2.3 Extraction and preservation of pulp/gel
2.4 Processing of apple, *Aloe vera,* development of jam, spread and other products
2.5 Low calorie sweeteners and their use in food products
2.6 Effect of storage on physico-chemical, nutritional and sensory quality of functional food products
2.7 Applications of Response Surface Methodology (RSM) for the optimization of product formulations

2.1 PRESENT STATUS OF *ALOE VERA* AND APPLE IN INDIA

India is one of the important fruit and vegetable producing countries in the world. Fruits and vegetables account for nearly 90 per cent of the total horticulture production in the country because of its agro climatic variations, enormous biodiversity, fertile soil and a large cultivable area. In fruit and vegetable production, it ranks second after China (Neeraj *et al.,* 2017; Wani and Songara, 2017). According to recent estimates, during 2018-19 the total annual production of fruit crops was 96,754 thousand metric tonnes from an area of 6,530 thousand hectare. Whereas, annual production of vegetable crops was estimated to be 1,87,474 thousand metric tonnes from an area of 10,436 thousand hectare (NHB, 2018). In Himachal Pradesh, total production of fruit crops and vegetables was 571.23 thousand metric tonnes and 1805.37 thousand metric tonnes from an area of 233.41 and 88.367 thousand hectare, respectively (NHB, 2018).

Apple (*Malus* × *domestica* Borkh) which belongs to family Roasaceae, is an important horticulture crop produced in temprate regions of the world (Rana and Bhushan, 2016). In India, apple is mostly grown in Jammu & Kashmir, Uttar Pradesh, Uttarakhand, Arunachal Pradesh and Himachal Pradesh. About 60 per cent of apple is supplied from J&K and 30 per cent from Himachal Pradesh and rest comes from other states. It is a fourth major fruit crop of India in terms of economic value. Apple production in India during the year 2018-19 was 2,371 thousand metric tonnes from an area of 307 thousand hectare. However, the annual production of apple in Himachal Pradesh was 4,46,570 metric tonnes from an area of 1,12,630 hectare (NHB, 2018).

Aloe vera is widely used for its healing and restorative properties. It belongs to the Asphodelaceae (Liliaceae) family and has thick green leaves which contain gel and latex. The gel consists primarily of water and the rest includes various vitamins, amino acids, enzymes, hormones, minerals and sugars, most of which are found in the human body. *Aloe vera* is widely used in the food industry, cosmetic and the pharmaceutical industry. The market has been growing steadily over the last decade driven by varied and increasing usage of *Aloe vera* in the food, health care and cosmetic industries. *Aloe vera* is a spiky, succulent, perennial plant which is grown in warm dry regions. It is popularly grown as indoors plant and cultivated almost everywhere in the world, both as a houseplant and for its medicinal qualities. There are about 300 identified species (Chandegara and Varshney, 2013). According to IASC (International Aloe Science Council) report the total worldwide cultivation of *Aloe vera* accounts 23,600 hectares of which the area included by America accounts 19,100 hectares. In India, *Aloe vera* is cultivated in Alwar (Rajasthan), Satanapalli (Andhra Pradesh), Rajpipla (Gujarat) and some parts of Tamil Nadu. About 200 tonnes of *Aloe vera* extract is used by pharmaceutical industries in India (Samsai and Praveena, 2016).

2.2 PHYSICO-CHEMICAL COMPOSITION, FUNCTIONAL AND NEUTRACEUTICAL PROPERTIES OF APPLE AND *ALOE VERA*

2.2.1 Apple

Apple (*Malus* × *dosmestica* Borkh) is one of the most popular fruit of temperate regions which is eaten as both raw and processed form such as apple juices, jams, cider, dried rings and juice concentrates. Apple is having high therapeutic and functional properties because it contains high level of antioxidants (quercetic and α-tocopherol) and phenolic compounds (chlorogenic acid and gallic acid) (Kalinowska *et al.*, 2014). The famous proverb *"An apple a day keeps the doctor away"* is highly recommended for maintaining the healthy diet among the people to stay fit due to high functional value of apple bioactive compounds (Francini and Sebastiani, 2013). The physico-chemical composition of apple fruit have been shown in the Table 2.1

2.2.1.1 Fruit size and weight

The weight and size of fruit generally depends upon the grade of fruits. Sharma *et al.* (2006) have reported mean weight of 80.20 g in minimum sized Golden Delicious apple fruits. Whereas, Raj *et al.* (2011) have reported average weight of 181.5 ± 10.71 g in apple fruit. According to Geddeda and Belal (2014), the average weight of Golden Delicious apples was 112.10 g. However, Kotiyal *et al.* (2017) in their study observed mean weight of 170.12 g in Royal Delicious variety of apple. Further, Kumar *et al.* (2018) and Verma *et al.* (2018) have reported the average weight of Golden Delicious apples to vary between 124.26 to 133.40 g.

Kheiralipour *et al.* (2008) has recorded fruit length and width of 75.28 mm and 84.12 mm in apple fruits, respectively. While, according to Raj *et al.* (2011) the average length and breadth of apple fruit was 70.5 ± 4.2 mm and 73.1 ± 3.2 mm, respectively. Whereas, fruit diameter of 3.20 cm and length of 5.23 cm have been observed in Golden Delicious apples by Geddeda and Belal (2014). According to Kotiyal *et al.* (2017), the average length and diameter of Royal Delicious apple fruit ranged from 44.24 mm to 53.87 mm and 50.53 mm to 74.73 mm, respectively. Further, Kishor *et al.* (2017) noticed that the average diameter and length of Golden Delicious apples was 59.63 mm and 66.17 mm, respectively. Almost similar findings for length (65.65 mm) and diameter (60.51 mm) in Golden Delicious apples were also represented by Verma *et al.* (2018).

2.2.1.2 Moisture content

Sharma *et al.* (2006) have reported range of 83.70 to 86.30 per cent moisture content in apple fruit. While, Mukhtar *et al.* (2010) reported that the moisture content of apple fruit was approximately 86.42 per cent. Further, average value of 83.69 and 84.93 per cent moisture content has been reported by Chakespari *et al.* (2010). In an another study, apple fruits have been found to contain 85.5 ± 0.45 per cent moisture content by (Raj *et al.*, 2011), while 85.30 per cent moisture content was observed by Ferretti *et al.* (2014). However, Khan and Hussain (2014) recorded mean moisture content of 87.7 per cent in Golden Delicious apples. Similarly, Khan *et al.* (2017) also found the moisture content of 85.03 per cent in apple fruit.

2.2.1.3 Titratable acidity

Ghafir *et al*. (2009) and Jha *et al.* (2012) have reported the titratable acidity of apple fruit ranged from 0.08 to 0.16 per cent. Whereas, Yosef and Belal (2014) reported that total acidity in Golden Delicious was higher (0.39 %) than Royal Starking (0.26 %) cultivar of apple. While, mean titratable acidity of apple fruit has been observed as 0.40 per cent by Kotiyal *et al.* (2017). However, slightly higher titratable acidity of 0.64 per cent has been recorded in Golden Delicious apples by Verma *et al.* (2018). Kumar *et al.* (2018) have reported the titratable acidity of 0.54 per cent (as malic acid) in Golden Delicious apple fruit.

2.2.1.4 Total soluble solids (TSS)

Jha *et al.* (2012) found the TSS in different apple cultivars ranged from 12.3 to 13.2 ºB. Whereas, Khan and Hussain (2014) observed 10.5 ºB TSS in Golden Delicious apples. In another study, TSS has been recorded around 18.40 ºB in apple fruit by Geddeda and Belal (2014). However, total soluble solids of 13.20 ºB have been reported in Red Delicious apple by Kotiyal *et al.* (2017). Likewise, different values of TSS in apple fruits as 12.27 ºB, 15.04 ºB and 9.98 ºB have also been reported by Kishor *et al.* (2017), Khan *et al.* (2017) and Verma *et al.* (2018), respectively.

2.2.1.5 pH

Chakespari *et al.* (2010) have reported a pH value of 4.20 in fresh apple fruit. Whereas, Khan *et al.* (2013) observed the pH range between 3.0 to 3.4 in different apple cultivars. In another study, pH value of 3.51 has been reported by Leahu *et al.* (2013).

However, pH value ranging between 3.05 to 4.21 in fresh apple fruit have been reported by different researchers and their co-workers (Hussain *et al.,* 2014; Khan and Hussain, 2014; Khan *et al.,* 2017).

2.2.1.6 Ascorbic acid

Mishra (2011) has recorded the highest and lowest value of ascorbic acid content in apple fruits as 10.42 mg/100 g and 3.75 mg/100 g, respectively. While, Lee (2012) reported 5.7 mg per 100 g of ascorbic content in apple fruits. Verma *et al*. (2018) observed ascorbic acid in Golden Delicious as 7.02 mg per 100g. However, the ascorbic acid content in different cultivars of apple fruits ranging between 6.07 to 7.50 mg/100g has been reported by Kotiyal *et al.* (2017). In another study, Kumar *et al.* (2018) have reported the ascorbic acid content of 21.60 mg per 100 g in Golden Delicious apple fruits.

2.2.1.7 Ash content

Guine *et al.* (2009) have reported the ash content in apple fruits ranging between 1.5 per cent to 3.9 per cent. Whereas, Campeanu *et al.* (2009) reported 1.81 per cent ash content in Golden Delicious apple fruit. Mukhtar *et al.* (2010) recorded the ash content to vary between 1.10 to 1.70 per cent. Whereas, Khan and Hussain (2014) reported the ash content of 0.29 per cent in Golden Delicious apple. However, mean ash content of 0.34 was recorded in apple fruit by Khan *et al.* (2017). According to study conducted by Okokon and Okokon (2019), the ash content of 0.3 per cent in apple fruit juice was reported.

2.2.1.8 Sugars

Chauhan (2001) observed that Starking Delicious apple contained about 6.09 per cent reducing sugars which increased during the growth and development phase of the fruit. While, Ali *et al.* (2004) observed the reducing sugar of 10.15 per cent in apple fruits. A wide range of total sugar content of 9.50 and 15.03 per cent has been reported by Campeanu *et al.* (2009) and Nour *et al.* (2010). Whereas, Lee (2012) recorded 12.39 per cent total sugar and reducing sugar content of 7.89 per cent in Golden Delicious apple fruit. Kotiyal *et al.* (2017) analyzed the mean value of total sugar in Red Delicious as 53.87 per cent. While, Kishor *et al.* (2017) have reported total sugar and reducing sugar content as 8.81 per cent and 7.90 per cent in Golden Delicious apple fruit. Similarly, Verma *et al.* (2018) have reported the total sugar content of 9.58 per cent and average reducing sugars of 6.04 per cent in Golden Delicious apple fruits.

2.2.1.9 Antioxidant activity

Duda-Chodak *et* al. (2011) found the antioxidant activity range between 31.78 to 60.03 mg per 100 g in apple fruit. Mishra (2011) has reported 74.79 per cent antioxidant activity in apple fruit. While, Jelodarian *et al.* (2012) observed the antioxidant activity of 63.92 per cent in apple fruits. In another study, the antioxidant activity of 33.67 (mMTE/L) in Golden Delicious apple was observed by Kishor *et al.* (2017). While, Kumar *et al.* (2018) have reported the antioxidant activity of 6.45 ± 0.06 micro mole Trolox per gram in apple fruit.

Table 2.1 Physico-chemical composition of apple fruits

Attributes	Values	Sources
Energy value (Kcal/100g)	54.0-59.0	Lee (2012); Ferretti *et al.* (2014); Khan and Hussain (2014); Liaudanskas *et al.* (2014);Bondonno *et al.* (2017); Khan *et al.* (2017); Kishor *et al.* (2017); Kumar *et al.* (2018); Verma *et al.* (2018).
Moisture content (%)	83.9-87.7	
Weight (g)	133.40-134.84	
Titratable acidity (% malic acid)	0.41-0.54	
Total antioxidant (µmol/g)	6.45- 6.51	
Total phenol (mg per 100g)	66.20 - 211.90	
Total carotenoid (µg/100g)	100.02-103.08	
Total sugars (%)	9.58-12.34	
Reducing sugars (%)	6.04-10.15	
Non-reducing sugars (%)	2.47-3.36	
Vitamin C (mg/100g)	7.02-21.60	
Mineral (%)	0.30-0.34	
Total soluble solids (ºB)	10.50-15.04	
Protein (%)	0.05-0.19	
Carbohydrates (%)	12.44 -15.3	
Fat (%)	0.13-0.36	
Fibre (%)	0.77-1.01	

2.2.1.10 Total phenols

Tzanakis *et al.* (2006) recorded the total phenols in green apple and red apple as 2,580 mg per 1000 g and 3,090 mg per 1000 g, respectively. Bonarska-Kujawa *et al.* (2011) have analyzed the total phenols of 56.59 per cent in apple fruits. Whereas, Hyson (2011) reported a wide range of total phenolic content ranging from 52.30 to 272.40 mg per 100 g. Further, total phenols of 39.88 mg per 100 g were reported in Golden Delicious by Lee (2012). While, Liaudanskas *et al.* (2014) analyzed the chlorogenic acid of 0.69 – 2.23 mg/g in apple fruit. A wide range of total phenolic content in apple fruits ranged between 66.20 to 211.90 mg per 100 g was given by Bondonno *et al.* (2017).

2.2.2 *Aloe vera*

Aloe vera (syn. *Aloe barbadensis* Miller) is an important and traditional medicinal plant belonging to the family Asphodelaceae (Liliaceae). *Aloe vera* is known by several names such as Ghrit Kumari, Kunvar pathu and Indian Aloe and is widely cultivated in dry areas because of its wide adaptability (Samsai and Praveena, 2016). The leaves contain more than 75 nutrients and 200 bioactive compounds, mono-saccharides and complex long-chain sugars which provide its unique healing and immuno-stimulating properties (Mulik and Phale, 2009). According to several research works, *Aloe vera* in human diet improves the digestion and possesses other biological activities include promotion of wound healing, antifungal activity, hypoglycemic or antidiabetic effects, anti-inflammatory, anticancer, immunomodulatory and gastroprotective properties (Hamman, 2008; Chandegara and Varshney, 2013).

According to Samsai and Praveena (2016), the leaves of *Aloe vera* possess many medicinal properties and are used to treat fever, enlarged liver, spleen and other glands related problems. Further, *Aloe vera* juice is also being marketed nowadays as a source of nutrient supplement and even as a medicament (Ramachandra and Srinivasa, 2008). It has been utilized as a functional ingredient in foods, especially in the preparation of health drinks, beverages, milk, ice cream and yoghurt (Eshun and He, 2004; Ramachandra and Srinivasa, 2008; Manoharan and Ramasamy, 2013). The physico-chemical charecteristics of *Aloe vera* leaf have been summarized in Table 2.2 and discussed as under:

2.2.2.1 Size, shape and weight

Sasikumar *et al*. (2013) reported the average leaf weight of 89.3 to 112.4 g among different structures of leaf used. Azam *et al*. (2014) observed mean *Aloe vera* leaf weight of 429.0 g/leaf and gel weight of 158.5 g/leaf in samples collected from different locations of Rajasthan. Jain (2016) reported *Aloe vera* leaf weight to range from 584.50 g to 592.61 g. Whereas, Pandey and Singh (2016) recorded average length as 300 mm and breadth as 90 mm in *Aloe vera* leaves. In an another study, the mean length and diameter of fresh *Aloe vera* leaf as 267.7 ± 3.55 mm and 80.23 ± 3.90 mm, respectively was recorded. While, weight of leaf was noticed a range of 190.56 ± 3.18 g (Burang, 2018).

2.2.2.2 pH

International *Aloe vera* Science Council (1997) specified whole leaf *Aloe vera* gel with 3.5 to 4.7 pH and use in food products. Similarly, Chaisawadi *et al.* (2005) reported the average pH value of 4.5 in *Aloe vera* gel. Whereas, a wide range of pH value of *Aloe vera* gel from 3.5 to 4.7 is reported by Chandegara and Varshney (2013). In another study, Srikanth *et al*. (2017) recorded the mean pH of *Aloe vera* juice as 4.3. While, Burang (2018) analyzed the average pH in whole *Aloe vera* gel as 4.5.

2.2.2.3 Total soluble solids

TSS content in *Aloe vera* gel was found to vary between 0.56 to 0.62 ºB (Lad and Murthy, 2013). Sharma *et al*. (2015) have recorded the average TSS of pure *Aloe vera* juice as 2.1 ºB. Whereas, Kiran *et al*. (2017) observed the TSS of *Aloe* juice as 1.5 ºB. However, Burang (2018) recorded the mean TSS value of 2.02 in *Aloe vera* gel/pulp.

2.2.2.4 Ascorbic acid

Several researchers have extensively studied about the ascorbic acid content present in *Aloe vera*. Gautam and Awasthi (2007) analyzed the ascorbic acid content of 27.0 mg per 100 g in *Aloe vera* leaf powder. While, Chandegara and Varshney **(**2013) reported that ascorbic acid content of fresh *Aloe vera* gel ranged between 47-61 mg per 100 mL. Whereas, Shubhra *et al.* (2014) have reported 1.90 and 1.56 mg per 100 g ascorbic acid in *Aloe vera* gel and juice. In another study, Sharma *et al.* (2015) have reported the ascorbic acid range between 23.75 to 234.85 mg per 100 g in different *Aloe vera* beverages, respectively. Burang (2018) has analyzed the ascorbic acid content as 7.89 ± 0.15 mg per 100 g in the fresh *Aloe vera* gel.

2.2.2.5 Ash content

Chandegara and Varshney **(**2013) observed ash content of 0.25 per cent in *Aloe vera* gel. Hamid *et al.* (2014) have reported the ash content of 0.20 per cent in *Aloe vera* gel on fresh weight basis. While, Jain (2016) has reported the mean ash content in *Aloe vera* leaf and *Aloe vera* gel as 16.63 per cent and 22.30 per cent, respectively.

2.2.2.6 Titratable acidity

Miranda *et al.* (2009) have reported 0.07 per cent acidity (as % citric acid) in *Aloe vera* gel. Whereas, Sasikumar *et al*. (2013) analyzed the acidity of 1.2 per cent in *Aloe* gel.

While, Di-Scala *et al.* (2013) recorded the acidity of 0.06 per cent in *Aloe vera* gel. Jain (2016) has reported titratable acidity of 0.10 per cent in *Aloe vera* gel and of 0.08 per cent in pure *Aloe vera* juice. Burang (2018) has recorded the average titratable acidity of 0.25 per cent in fresh *Aloe vera* leaf.

2.2.2.7 Moisture content

Aloe vera contains higher amount of water content which is discussed by many workers. Hamman (2008) reported that the raw pulp of *Aloe vera* contains approximately 98.5 per cent water, while the mucilage or gel consists of about 99.5 per cent water. However, Boghani *et al*. (2012) have reported a moisture content of 96.31 per cent in *Aloe* gel. Ahmed and Hussain (2013) have noted the water content of 95 to 98.5 per cent in *Aloe vera* leaves. While, mucilage or gel consisted 97.95 per cent of moisture content as reported by Hamid *et al*. (2014). Radha and Laxmipriya (2015) observed that moisture content range from 99 to 99.5 per cent in *Aloe vera* gel.

2.2.2.8 Sugars

Jain (2016) reported that the average total sugars and reducing sugars as 0.72 and 0.20 per cent, respectively. However, according to Sangma *et al*. (2016), the total sugar content of 1.90 per cent was present in *Aloe vera* juice. Whereas, Chaudhary *et al*. (2017) recorded 1.02 per cent of reducing sugars and 2.10 per cent of total sugars in *Aloe* gel. Burang (2018) analyzed the mean total sugars and reducing sugar 1.02 per cent and 0.58 per cent in *Aloe vera* leaf, while 0.96 per cent and 0.32 per cent in *Aloe vera* gel, respectively.

2.2.2.9 Polysaccharides

Lad and Murthy (2013) have reported 0.19 per cent of polysaccharide concentration in gel and 0.17 per cent in juice on fresh weight basis. Whereas, Azam *et al.* (2014) recorded mean polysaccharide content of 0.26 per cent in *Aloe vera* gel. *Aloe vera* polysaccarides are derived from the mucilage layer of the plant and are known as mucopolysaccharides. The most prominent monosaccharide is mannose-6-phosphate, and the most common polysaccharides are called glucomannans (Quispe *et al.*, 2018).

2.2.2.10 Total phenols

Jain *et al.* (2014) found the significant linear correlation between total phenols and antioxidant activity of plant extract and they observed maximum amount of phenol present (0.019 mg GAE per g) in water extract of *A. saponaria* followed by acetone extract (0.015 mg GAE per g) and minimum in ethanol extract (0.010 mg GAE per g). Whereas, Jain (2016) recorded the total phenols of 0.31 mg per 100 g in *Aloe vera* gel and 0.23 mg per 100 ml in pure *Aloe vera* juice. Burang (2018) reported that the total phenols of 198.70 mg per 100 g were present in *Aloe vera* pulp and 220.7 mg per 100 g in whole *Aloe vera* leaf.

2.2.2.11 Antioxidant activity

Sharma *et al*. (2015) have reported 71.81 per cent antioxidant activity of pure *Aloe vera* juice. Taukoorah and Mahomoodally (2016) analyzed the antioxidant activity of pure *Aloe vera* gel and found it to be 72.42 ± 0.95mM Trolox Equivalent (TE). Hes *et al.* (2016) recorded the antioxidant activity in *Aloe vera* extract which ranged between 4.32–8.87 and 0.58–0.87 mg of trolox per 1.0 g dry matter. Burang (2018) has reported that antioxidant activity (% DPPH free radical scavenging activity) of *Aloe vera* leaf and *Aloe vera* pulp as 89.24 and 71.12 per cent, respectively.

Table 2.2 Physico-chemical composition of fresh *Aloe vera* leaf

Attributes	Values	Sources
Physical parameter		Chaisawadi *et al.* (2005); Hamman (2008); Ahlawat and Khatkar (2011); Chandegara and Varshney (2013); Lad and Murthy (2013); Sasikumar *et al*. (2013); Di-Scala *et al.* (2013); Radha and Laxmipriya (2015); Sharma *et al*. (2015); Jain (2016); Sangma *et al*. (2016); Chaudhary *et al*. (2017); Srikanth *et al*. (2017); Burang (2018);
Leaf size:		
a) Length (mm)	267.7-271.2	
b) Diameter (mm)	80.23-84.13	
Leaf weight (g)	89.3 to 112.4	
Pulp recovery (%)	34.7-45.4	
Chemical parameters		
Energy value (Kcal/g)	5.84-5.93	
Moisture (%)	99-99.5	
pH	4.5 -4.62	
TSS (ºB)	0.56-2.16	
Titratable acidity (%)	0.096-1.20	
Ascorbic acid (mg/100 g)	8.04-61	
Total sugars (%)	0.72-1.90	
Reducing sugars (%)	0.32 - 1.02	
Total phenols (mg/100g)	198.70 - 222.1	
Antioxidant activity (% DPPH free radical scavenging activity)	13.47-71.81	

2.3 EXTRACTION AND PRESERVATION OF JUICE/ PULP

2.3.1 Apple and Other Fruits

Shakir *et al.* (2008) have extracted apple pulp after washing, sorting, peeling and coring the fruit pieces with the help of stainless steel knife. Then pieces were dipped in 0.2 per cent citric acid solution and fruit pulp was extracted by using cold pulping method. According to a method given by Arampath and Dekker (2019), fresh mangoes were washed, peeled and cut into pieces and passed through a pulping machine for pulp extraction. Pulp separated from the machine was thermally treated at 85 ± 1 ºC for 20 to 25 min and preserved in the plastic container by adding sodium metabisulphite (50–60g/60 kg pulp) as preservative. Whereas, Raj *et al.* (2011) prepared the apple juice by washing, grating the fruits followed by juice extraction by using hydraulic press. Further according to Sharma *et al*. (2013), apricots were heated for 5-7 minutes in a stainless steel pan by adding water (100 mL/kg of fruit) and passed through the pulping machine for extraction of pulp. Apricot pulp was collected and packed in the barrels for further experimentations.

However, Xu *et al.* (2018) suggested a method of tomato pulp extraction by using hot break method. Pulp was extracted by cutting the fruit into two halves followed by cooking (90 ºC) in the steam jacketed kettle for two minutes and homogenized before packaging. In an another study, apple juice was extracted by dipping the grated shreds into a solution containing 0.5g ascorbic acid per kg. The juice was expelled out in hydraulic press with 5 MPa pressure for 5 min. Extraction of apple juice with the help of hydraulic press for further utilization in product development (Osteke *et al.,* 2014; Hassan *et al.,* 2015; Lal *et al.,* 2015; Sharma *et al.,* 2019).

2.3.2 *Aloe vera*

According to the extraction method given by Chang *et al.* (2006) and Hamid *et al.* (2014), the *Aloe vera* leaves were washed and cutting from the sides. Then the parenchyma cells (gelatinous layer) were separated from the epidermis (skin) and washed the fillet with distilled water to remove exudates from their surface. Further, the fillets were cut into cubes and blended in food processor and squeezed through a 200-mesh screen. While, in a study, Chandegara and Varshney (2013) reported the additional step of charcoal addition to decolourize the *Aloe vera* gel prior to preservation. According to Abid and Zahid (2016), gel was separated by hand filleting method which was reported to get maximum quantity of gel and to ensure that the final product/blend contains all the nutrients that are initially present in the *Aloe vera* leaf. Whereas, Jaddu *et al*. (2016) reported the pre-treatment (blanching) prior to juice extraction where the leaves were blanched for 3-5 min at 92 ºC for the inactivation of deteriorative enzymes as well as softening the tissues and to reduce the viscous nature of *Aloe vera* gel. Mulik and Phale (2009) have reported that the gel was extracted by using heat treatment (70 ºC) followed by purification of gel. Whereas, some researchers extracted the *Aloe vera* juice by using a modified cold extraction method. They homogenized the gel by passing through a grinder and enzymatically treated (@ 0.5 % pectinase) followed by filtration and pH adjustment (3.5) by addition of citric acid. Then 0.5 per cent of ascorbic acid was added as anti-browning agent and the juice was hot filled in pre-sterilized bottles (Sharma *et al.*, 2015).

2.4 PROCESSING OF APPLE, *ALOE VERA*, DEVELOPMEMT OF JAM, SPREAD AND OTHER PRODUCTS

Fruits and vegetables play a significant role in human nutrition and provide us a range of phytochemicals, many of which have more than one role, being involved the protection against diseases, such as heart cancer, stroke and diabetes (Ramjan and Kumar, 2019; Sharma *et al.,* 2019). Fruits and vegetables are perishable in nature containing different constituents such as water, carbohydrates, fats, proteins, fiber, minerals, organic acids, pigments, vitamins and antioxidants, among others (Kader *et al.,* 2014). However, due to perishable nature of fresh fruits and vegetables, their immediate utilization in raw or value added form is very important. Value addition is the process of taking a raw commodity and changing its form to produce a high quaility end product. Whereas, value added is defined as the addition of time, place, and/or form utility to a commodity in order to meet the tastes/preferences of consumers (Pant and Chinwan, 2014). The whole fruits and vegetables can be successfully converted into dehydrated products, beverages and intermediate moisture food like jams, spread, bars, toffee etc (Hymavathi, 2018).

Apple and *Aloe vera* contains high nutraceutical and therapeutic components. *Aloe vera* is also having antiseptic and antibacterial properties which are very useful for the development of product without the addition of preservative. Therefore, its utilization in the production of food products is very effective approach to meet healthy wellbeing of human. There was very less work available on the incorporation of *Aloe vera* because of its bitter taste and lack of knowledge about its functional nutrients. But, from the last few decades, scientists started working on its utilization and also started exploring its medicinal components. Even, no scientific work was available on apple spread and *Aloe vera* fortified apple spread. In the present study, efforts were made for the first time in the country to develop a palatable and nutritious product with reduced calorie for the benefit of consumers.

Many researchers utilized the fruits for the development of different types of food products such as spreads, jams, jellies, RTS, juices etc. Fruit spreads are jam like products which provides smooth consistency and spreadability. Fruit spreads are manufactured similar to jams and jellies, which are made with various fruits preserved by adding sugar and thickened to a certain extent by application of heat. Whereas, according to Food and Agriculture Organization (FAO) and World Health Organization (WHO), fruit spreads differ in terms of degree of gel formation and ingredient composition, which makes it an excellent alternative for the jams due to its reduced sugar of 25 to 50 ºB composition and its generally high percentage of fruits containing vitamins and minerals.

According to Holzwarth *et al.* (2013), strawberry spread was prepared by mixing the fruit pulp with sucrose (1722 g), glucose (450 g) and water (300 ml). Then the mixture was heated under reduced pressure and temperature (85 ºC) until TSS of 42 ºB was achieved with respect to improve taste, colour and spreadability (Peinado *et al.,* 2013; Barcelon *et al.* 2015). Barcelon *et al.* (2015) have reported that the production of fruit spreads from white and red dragon fruit followed by cooking of fruit pulp at 70 ºC along with citric acid and sugar with continuous stirring until the desired smooth texture was obtained. Then the cinnamon powder and vanilla extract were added and packed immediately in pre sterilized jars. Final products were evaluated for similar sensory score as compared to the commercial strawberry spread. In another scientific study, some workers prepared the fruit spread from banana and carrot. Then they were steam blanched (10 min) and blended by using home blender for the preparation of puree. The whole mixture was cooked along with sugar, citric acid as well as carboxymethyl cellulose, guar gum and xanthan gum as thickening agent. The mixture was homogenized at the speed of 6000 rpm for 3 minutes followed by cooking and stored under refrigerated conditions and further subjected to the analysis of rheological properties (Sorour *et al*, 2016).

However, Shah *et al.* (2015) blended apple pulp with olive pulp in 100:0, 90:10, 80:20, 70:30, 60:40 and 50:50 ratio, respectively for the preparation of mixed fruit jam. They observed that the ratio of 60:40 and 50:50 were the most effective jams according to sensory analysis. Dahiwale and Dhurve (2017) have studied about the preparation of guava jam blended with carrot and basil extract. The guava pulp, carrot and basil extract were used for the preparation in the ratio of 55:40:5, 45:50:5 and 40:55:5 respectively. They observed that blend of 55:45:5 got higher sensory score for colour (9), flavor (9), consistency (8), taste (8) and overall acceptability (9).

Aloe vera gel is considered as very important ingredient in Ayurveda due to its medicinal potential, which was further used by researchers in the formulations of functional food products as described in Table 2.3. Ahmed *et al*. (2016) studied the neutraceutical function of *Aloe vera* and prepared a health drink by blending *Aloe vera* and moringa leaf extracts in different proportion. They found that the blend containing *Aloe vera* and moringa in the ratio of 70:50 was the best based on the highest sensory score. Hossain *et al*. (2017) prepared functional ready-to-serve (RTS) beverage by blending jackfruit and *Aloe vera* juice in varying formulations such as 100:0, 90:10, 80:20, 70:30 and 60:40 to improve the flavour and therapeutic properties, out of which the 70:30 blend obtained the highest sensory score for overall acceptability and further they concluded that *Aloe vera* juice could be successfully mixed up to 30 per cent with the jackfruit juice for the development of blended ready to serve beverages. Vikram and Sikarwar (2018) developed *Aloe vera* fortified kinnow - aonla squash. They have used the kinnow-aonla juice along with *Aloe vera* juice for the preparation of functional squash. Kinnow and aonla juice was left for 24 hours in the refrigerator to settling down and mixture of juice was heated at 96 ºC for two minutes to inactivate enzymes. Then sterilized *Aloe vera* juice was mixed with the kinnow and aonla juice. The concentrations of sugar (50 ºB) and citric acid (1.0 %) were constant for all the formulations which were boiled in water. Then filtered the syrup and mixed the juice along with 350 ppm KMS in the final products. The treatment combination of 25 % juice blend (consisting 5 % kinnow + 70 % aonla juice + 25 % *Aloe vera* gel) was found to be the most suitable in terms of quality and sensory scores.

Whereas, different formulations of apple pulp and *Aloe vera* gel were developed by Kanojia *et al.* (2018) for the preparation of jam. They have used three different concentrations of sugar such as 1 per cent, 1.5 per cent and 2 per cent along with the different ratio of apple and *Aloe vera* pulp (80:20, 60:40, 40:60 and 20:80) for increasing the nutritional and medicinal value of the prepared functional jam. It was found that the treatment with the ratio of apple : *Aloe vera* (40:60) along with 1 per cent sugar was most effective jam blend because of minimum change in moisture content (45.95 to 45.98 %), protein (5.09 to 5.06 %), carbohydrates (25.89 to 27.85 %), ash content (5.35 to 5.27 %) and pH (4.26 to 4.34).

Whereas, Rahman *et al.* (2015) utilized the fruit pulp of ripe mango and pineapple fruits for the preparation of the jam at different levels such as ratio of mango pulp : pineapple pulp : *Aloe vera* gel (50:20:30, 40:40:20, 60:20:20, 40:50:10) along with constant sugar and acid concentation. Out of which 40:40:20 had better overall acceptability score. Ullah *et al.* (2018), prepared the apple and carrot jam in six different batches. Carrots and apples were washed, peeled and slices of apple were dipped in water containing 1 per cent citric acid to avoid oxidation while carrots were boiled in water for softness. The pulp was extracted by pulping machine and all batches were cooked to a final consistency of the jam. They described that the formulation of apple and carrot pulp (60 % + 40 % and 50 % + 50 %) were preferred by judges (Table 2.3).

Garrido *et al.* (2014) used the apple juice extract for the preparation of apple jelly. They found the best formulation of juice (350 g), sugar (550 g) and pectin (5g/kg) for the best rheological properties of final product. Palve *et al.* (2015) have developed the pine apple jelly with the incorporation of *Aloe vera* in different concentrations. They have prepared the fruit jelly by cooking pine apple juice and *Aloe vera* gel with sugar, pectin and 0.7 per cent of citric acid until the desirable consistency. They observed that the ratio of pineapple : *Aloe vera* (60:40) had better physico-chemical and overall acceptability score. Kumar and Deen (2017) developed wood apple jelly with 75 per cent water and 25 per cent mature fruit pulp was found to be best for better pectin extract recovery and the jelly prepared from the extract was found to be best during organoleptic quality.

Table 2.3 : Food applications of *Aloe vera*

Sr. No.	*Aloe vera* products	Food application	Product composition	References
1.	Gel	a) Fruit coatings	a) 15 per cent *Aloe vera* was mixed with 5 per cent glycerol and 0.1 per cent almond oil	Mahmoud *et al.* (2019)
2.	Juice	a) *Aloe vera*-bael RTS b) Yoghurt c) *Aloe vera*-mango nectar d) *Aloe vera*-aonla squash	a) 75 per cent bael pulp and 25 percent *Aloe vera* gel b) Cow milk with the addition of 3 per cent sugar and 14 per cent *Aloe vera* juice c) Mango pulp with added sugar 15 per cent TSS and 20 per cent *Aloe vera* juice d) 65 per cent *Aloe vera* juice + 35 per cent aonla juice with 30 per cent juice part and 40°B TSS	Tiwari and Deen (2015) Mukhekar *et al.*, (2018) Elbandy *et al.* (2014) Sharma *et al.* (2018)
3.	Powder	a) Ice cream b) Butter milk c) Capsule	a) 20 per cent *Aloe vera* powder containing 10 per cent fat and 36 per cent total solids b) 60 per cent curd with the addition of 30 per cent water and 10 per cent *Aloe vera* c) *Aloe vera* dietary supplements (0.33 to 750 mg /capsule)	Manoharan and Ramasamy (2013) Mudgil *et al.* (2016) Steenkamp and Stewart (2007)
4.	Concentrate	a) Jam b) Jelly	a) 40 per cent Pineapple, 40 per cent mango and 20 per cent *Aloe vera* b) 60 per cent Pineapple juice and 40 per cent *Aloe vera*	Rahman *et al.* (2015) Palve *et al.* (2015)

Abid *et al.* (2017) have worked on the preparation of multiple pomegranate based jam. They have mixed the pomegranate puree with half the quantity of sugar and stirred at room temperature until dissolution of sugar. Then 0.2, 0.7 and 1.2 per cent of low methoxylated pectin was added in the different treatments and the results revealed that treatment containing 0.2 per cent of low methoxyl pectin exhibits best organoleptic quality. Rahman *et al.* (2018) have used 50 per cent guava pulp with different proportions of pectin for the production of the guava jam. Then the pulp was cooked in the steam kettle along with other ingredients and they recorded best acceptability of two samples containing pectin, 1.0 per cent and 1.2 per cent, respectively.

Whereas, Mishra and Sangma (2017) have developed *Aloe vera* fortified RTS by blending with ginger, sweet lime and aonla in the ration of 40:5:40:15, 50:5:30:15, 60:5:20:15 and 70:5:10:15. They found that the RTS prepared by using 60:5:20:15 ratio obtained higher overall sensory acceptability. Lavanya *et al.* (2018) used the *Aloe vera*, guava and jamun pulp for the preparation of health drink (nectar). They have used different proportions of pulps (*Aloe vera*: Guava: Jamun) in the ratio of 2:2:16, 2:4:14, 2:6:12, 4:2:14, 4:4:12, 4:6:10, 6:2:12, 6:4:10 and 6:6:8 with different TSS level of 15 °B and 17 °B with 0.30 per cent acidity followed by heated at 95 °C for 5 min. Immediately after pasteurization, the health drink was packed in 200 ml pre-sterilized glass bottles followed by processing in boiling water for 30 min at 96 °C.

2.5 LOW CALORIE SWEETENERS AND THEIR USE IN FOOD PRODUCTS

In the last decades, growing concerns about health and quality of life have encouraged people to avoid the consumption of food rich in sugar, salt and fat (Siervo *et al.*, 2014). A large proportion of sugar is consumed from processed foods and therefore, World Health Organization recommends safe limits for the consumption of free sugar in the diet to less than 10 per cent of total energy intake. Due to the health implications of consuming a diet high in sugar, such as obesity, diabetes, and heart disease, food industry has reduced the use of sugar and substitute it with the ingredients that mimic the functional property of sugar (non-nutritive sweeteners that have lower or zero calories) (Jain and Grover, 2015; Pielak *et al.*, 2020). These sweetener plays an important role in providing flavor, appearance, color, taste, and dimension to the finished product. Due to prevalence of diseases like diabetes and obesity, the use of artificial sweeteners as a sucrose substitutes for the development of low-calorie products has been the focus of R&D in the recent past years (Aggarwal *et al.*, 2016;

Mansoor *et al*., 2017). Food products containing non-nutritive sweeteners (NNSs) instead of sugar have become increasingly popular. However, with the dramatic increase in their consumption, it is reasonable and timely to evaluate their potential health benefits and more importantly, potential adverse effects (Beereboom, 1979; Renwick and Molinary, 2010; Lohner *et al.* (2017). Non- nutritive sweeteners offer less than two per cent of the calorie value of sucrose per equivalent unit of the sweetening capacity (Chattopadhay *et al.,* 2014; Belloir *et al.,* 2017; Pielak *et al.,* 2020).

2.5.1 Sweetos

"Sweetos" is one of the low calorie sweeteners that contain the combination of fructo-oligosaccharide and sucralose. It is a liquid bulk sweetener, which has sweetness equivalent to that of sugar (sucrose). It means that on a weight-to-weight basis sweetos can be substituted for sugar. The compositional characteristics of sweetos are shown in the Table 2.4.

(a) Fructo-oligosaccharide: Fructooligosaccharides (FOS) are diverse group of carbohydrates which are manufactured from a mixture of kestose (39%), nystose (53%) and fructosyl nystose (7%), obtained from sucrose by enzymatic addition of fructosyl moieties, and also having an average degree of polymerization of 3.6 (Khanvilkar and Arya, 2015; Oku and Nakamura, 2017). Fructooligosaccharides (FOS) have half the calories per gram than sucrose or glucose with sweetness relative to sucrose of 0.3-0.6. FOS is present in more than 36,000 plant sources as reserve carbohydrates. It is present in various plant sources such as asparagus, jerusalem artichoke, chicory, sugar garlic, onion, wheat, honey, banana, barley, tomato, and rye (Sridevi *et al.,* 2014). The highest concentration is present in Jerusalem artichoke (up to 20 %) and chicory (between 5 to 10 %). FOS is also having many nutraceutical properties due to its high content of various minerals, vitamin C and B group vitamins (Garcia-Almeida *et al.*, 2013). FOS are well recognized prebiotics, which are non-digestible food components that beneficially affect the host health by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon (Romano *et al.,* 2016).

(b) Sucralose: Sucralose is prepared from either of two sugars (sucrose or raffinose). It is a high quality intense sweetener which is non-caloric, non-carcinogenic and has no effect on blood sugar level (Binns, 2003). It has obtained GRAS (Generally Recognized As Safe) status and is about 600 times as sweeter as sugar (sucrose). It is produced from sucrose when three chlorine atoms replace three hydroxyl groups (Ansari *et al.*, 2015; Choudhary, 2017). Unlike other artificial sweeteners, it is stable when heated and can therefore be used in baked and fried goods (Al-dabbas and Al-qudsi, 2012). About 15 per cent of sucralose is absorbed by the body and most of it passes out of the body unchanged. Further, the way sucralose is metabolized in the body has suggested a reduced risk of toxicity. The acceptable daily intake (ADI) for sucralose is 5-15 mg/kg body weight/day (Brahmini *et al.*, 2012). Due to its acceptable taste, heat stability and physico-chemical properties, it has become a very versatile sweetener suitable for use in a wide variety of food products such as soft drinks, desserts, ice creams, confectionery, preserves and sandwich spreads etc. (Dhartiben and Aparnathi, 2017).

Table 2.4: Physico-chemical properties of sweetos

Properties	**Value**
Appearance	Yellowish thick syrup
Total FOS (%)	56.91
Sucralose	Present
Taste	Sweet
Moisture (%)	17.31
TSS (ºB)	80
pH	5.49
Ash content (%)	<0.006
Energy value (Kcal/g)	1.54
Total plate count (cfu/ml)	<10

Source: Anonymous (2014)

2.5.2 Other Alternative Sweeteners

Acesulfame K, saccharine, stevia, aspartame, sorbitol, xylitol etc. are non-nutritive sweeteners which are marketed under different brand names (Ott *et al.*, 1991; Donnell and Kearsley, 2012; Garcia-Almeida *et al.,* 2013; Jain and Grover, 2015). These non-nutritive sweeteners (NNSs) are reported to be many times sweeter than sucrose and provide low calories to the consumers (Ceunen and Geuns, 2013; Ansari *et al.*, 2015; Jacob *et al*., 2016). So, these sweeteners have been used as an alternative to traditional sugar in wide variety of foods and beverages like dairy products, bakery products, medicine, sweets and chewing gum, jams, marmalades, preserve and canned fruit etc (Whitehouse *et al.,* 2008; Lean and Hankey, 2015; Dhartiben and Aparnathi, 2017).

2.5.3 Application of Non-nutritive Sweeteners for the Development of Low Calorie Products

Now-a-days, sugar free foods are very much popular because of their less calorie content. Artificial sweeteners/non-nutritive sweeteners are synthetic sugar substitutes but may be derived from naturally occurring substances, including herbs or sugar itself. The growing consumer interest in health and its relationship with diet has led to a considerable rise in the demand for low calorie fat products. So, food industry uses various non-nutritive sweeteners which are low in calorie content instead of high calorie sugar. Various scientists have suggested the use of artificial sweeteners/low calorie sweeteners for the development of different food products (Sharma *et al.,* 2003; Barwal *et al.,* 2005; Sharma and Joshi, 2015; Dhartiben and Aparnathi, 2017; Philip and Peter, 2018; Pielak *et al.,* 2020).

Muhammad *et al.* (2008) developed low calorie apple jam using non-nutritive sweeteners in the combination of aspartame with cyclamate (2.08 + 12.5 g/kg pulp) and aspartame with saccharine (2.08 + 1.25 g/kg pulp). They observed that the apple jam contained non-nutritive sweetener, aspartame and cyclamate (2.08 + 12.5 g/kg pulp) was preferred by judge. However, Kamal *et al.* (2015) prepared an apricot diet jam by replacing 100 per cent sucrose with saccharine and aspartame. They have reported the best quality jam with results of chemical analysis as pH (3.69), total acidity (0.66 %), vitamin C (6.54 mg/100 g), moisture (77.01 %), TSS (21.3 ºB), reducing sugars (4.13 %), and non reducing sugars (9.2 %).

Guggisberg *et al.* (2011) standardized recipe of low-fat and whole milk set yoghurt by replacing 8 per cent sugar with stevia, with a combination of actilight and stevia (2 to 6 %) in a set yoghurt. Skimmed milk with sucrose or sweetener was heated at 92 ºC and fermented at 42 ºC until a pH of 4.6 was attained. They concluded that 6 per cent actilight together with stevia had best organoleptic properties. Whereas, Pugazhenthi and Jothylingam (2013) prepared low calorie herbal flavoured milk for enhancing the functional properties by incorporating *Aloe vera* pulp extract at different concentrations (3 %, 5 % and 7 %) and artificial sweeteners like aspartame and sucralose at different levels of sugar replacement. The study revealed that dietetic herbal flavoured milk up to 75 per cent replacement of sugar with aspartame and 100 per cent replacement of sugar with sucralose were found to be ideal for almost all sensorial qualities as well as slight increase in mineral content due to addition of *Aloe vera*.

Youssef and Mousa (2012) prepared low calorie baladi rose petals jam by using sorbitol. They prepared the three types of jam named as: fresh baladi rose petal jam with 40 per cent sugar, fresh baladi rose petal jam with 40 per cent sorbitol and dried baladi rose petal jam with 40 per cent sorbitol. Both the products (fresh baladi rose petal jam and dried baladi rose petal jam) were got high scores in the sensory evaluations. Whereas, Basu *et al.* (2013) have prepared low calorie mango with the replacement of table sugar with non-nutritive sweeteners like stevioside and sucralose in different ratios viz. 0, 25, 50, 75 and 100 per cent. They observed that mango jam with 25 per cent stevioside and sucralose substitution was recorded as high score in the sensory evaluation.

However, Alizadeh *et al.* (2014) have used stevia as a substitute for sugar on the preparation of fruit based milk shake. Five different treatments of fruit milk shakes were prepared with sucrose/stevia ratios of 100:0, 75:25, 50:50, 25:75 and 0:100. The recommended ratio of sucrose/stevia in beverage for the best sensorial acceptance was 25:75. It was concluded that stevia was a good choice to develop low sucrose beverages including fruit based milk shakes. Although, stevia had no adverse impact on physico-chemical properties, its sensorial acceptance is affected by its rate of addition.

Viktorija *et al.* (2013) have produced plum jams with different sweeteners like fructose, sorbitol and agave syrup. All the jams were evaluated superior in terms of overall acceptability and found lower vitamin C content from 11.84 mg/100 g to 12.3 mg/100 g as compared to the fresh plum (14.5 mg/100 g). Abolila *et al.* (2015) have developed orange based low- calorie jam by replacing sugar with fructose, stevioside and sucralose and found that the jam sweetened with 50 per cent fructose, 33.50 per cent sucralose and 16.50 per cent stevioside was found best on the basis of sensory quality characteristics. Whereas, Alsuhaibani and Al-Kuraieef (2018) prepared different low-calorie pumpkin jams fortified with soybean where sucrose was replaced with fructose, stevia and aspartame sweetener. Different jam formulations were prepared to select the best proportion of sweetener such as high fructose corn syrup (625 g), stevia (4 g) and aspartame (25 g). It was observed that the jam sweetened with high fructose corn syrup was liked more in the sensory evaluation.

However, Sharma and Tandon (2015) developed antioxidant rich low calorie functional bitter gourd spiced squash through fortification with ascorbic acid and using stevioside sweetener at different proportions (25, 50, 75 and 100%). They concluded that the caloric value of the product was reduced up to 60 per cent at 75 per cent stevioside sweetness as compared to the standard control sample.

In another study, the low calorie diet biscuits were prepared by Aggarwal *et al.* (2016) with the help of sweetos (fructo-oligosaccharide + sucralose). They have used the formulation of multigrain flour containing dairy ingredients to prepare dairy–multigrain flour. The maximum proportion of sugar was replaced with the blend of FOS-sucralose (5.75 %) and maltitol (17.25 %) without affecting the sensorial perception. The study demonstrated that highly acceptable reduced-calorie biscuits can be produced by using dairy–multigrain composite flour with maltitol and FOS-sucralose (3:1). While, Sharma *et al*. (2018) prepared a low calorie beverage by blending *Aloe vera* with aonla juice level containing 90 per cent stevioside and 10 per cent sorbitol sweetness proportion which was highly scored by the sensory analysts. The developed product showed strong antimicrobial activity (28.50 mm inhibition zone) against *E. coli* as well as high antioxidant potential (66.90 %) and a low energy value was calculated as compared to the control product.

Belovica *et al.* (2017) have worked on the development of low calorie jams with increased content of natural dietary fibre prepared from tomato pomace. Four jam formulations were developed, starting with the basic formulation containing sucrose and without added pectin. In the second and third formulation sucrose was partially (50 %) replaced by stevioside while in fourth formulation sucrose was completely replaced by fructose and stevioside. Third and fourth formulations were assessed by the sensory panel as more spreadable and more acceptable jams. Banas *et al.* (2018) have prepared two types of a prebiotic low calorie gooseberry jam with the addition of inulin and steviol glycoside. The jam was prepared with addition of wheat germ and defatted flex seed powder. Moreover, the partial replacement of sucrose with steviol glycoside and inulin enables energy value reduction in such jams. While, Sutwal *et al.* (2019) prepared low calorie apple jam by using different concentration of stevia (0.15 %, 0.3 %, 0.45 % and 0.6 %) in the place of sugar. Fruit pulp along with all the ingredients (except stevia extract) was cooked properly up to the final consistency of jam and then stevia extract was added at the end. Among all the samples, jam recipe containing 0.6 per cent stevia extract showed highest sensory score for all the parameters (sweeteness, colour, taste and overall acceptability).

Pielak *et al.* (2020) prepared a low calorie apple preserve by using steviol glycosides along with sugar. They have substituted 1 g of sugar with 0.01 g of steviol glycosides at 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 percent levels for the preparation of low calorie preserve. They concluded that the consumer acceptability for sugar substitution with 40 per cent (0.20 g/100 g) steviol glycosides was high in the preserve and they have discussed that the higher level of substitution (more than 40 %) led to metallic flavor, bitter taste, astringent oral sensation, and a sharp odor in the fruit preserves. In an another study, Sharma *et al.* (2020) developed fructooligosaccharide fortified low calorie apple-whey based RTS beverage which contained 75 per cent apple juice + 25 per cent whey with 2.5 per cent *jaljeera* extract and 13 ºB TSS. Results revealed that the beverage with 75 per cent sweetos (mixture of fructooligosaccharide and sucralose) was found to be most acceptable with overall acceptability score of 8.59 ± 0.26.

2.6 EFFECT OF STORAGE ON PHYSICO-CHEMICAL, NUTRITIONAL AND SENSORY QUALITY OF FUNCTIONAL FOOD PRODUCTS

Storage has important effects on various physico-chemical and organoleptic attributes of the food products. Storage time and conditions are the most important factors for significant changes in the food products. Various researchers have studied the effect of storage duration on nutritional, biochemical, microbiological and sensory attributes of the fruit products and discussed below under different heads:

2.6.1 Moisture Content

Muhammad *et al.* (2008) reported that the moisture content decreased in two samples of apple jam from 84.00 per cent to 75.42 per cent, 70.00 per cent and 68.00 per cent during storage, respectively. Whereas, Karpagavalli *et al.* (2014) found 1.00 per cent moisture increase in the jackfruit jam during 6 months storage. Rahman (2018) has recorded the initial moisture content of strawberry jam ranged from 25.78 to 29.67 per cent in the samples T_3 and T_1, respectively, and after 2, 4 and 6 months of storage the moisture content slightly increased , which might be due to moisture absorbed by sugar component of jam. At the end of the storage period moisture content was highest in T_1 (29.93 %), while the lowest in T_3 (26.11 %). After 6 months of storage the amount of gained moisture content was 0.88 per cent in T_1 and 1.28 per cent in T_3. Whereas, Sutwal *et al.* (2019) have observed the moisture content of the apple jam in the storage period of 28 days. Initially, the moisture content was recorded as 34.00 per cent which reduced to 32.45 per cent during storage.

2.6.2 Titratable Acidity

Touati *et al.* (2014) have recorded the titratable acidity in apricot jam before storage as 0.82 per cent which significant increased to 0.98 per cent, 1.01 per cent and 1.03 per cent at 5 ºC, 25 ºC, and 37 ºC, respectively. However, Kumar and Deen (2017) have recorded increase in titratable acidity in wood apple jelly of six months storage. They recorded the acidity increased from 0.57 per cent to 0.77 per cent. Whereas, Rahman *et al.* (2018) observed a significant increase in the acidity from 0.72 to 0.89 per cent of guava jam during 90 days storage under ambient conditions. Brandao *et al.* (2018) have also observed significant increase in the titratable acidity from 0.41 to 1.2 per cent in mixed fruit jam during storage period of 140 days.

2.6.3 Total Soluble Solid

Muhammad *et al*. (2008) have developed diet apple jam using different non-nutritive sweeteners and reported that TSS of diet jam significantly increased from 11.54 to 17.70 during 90 days of storage. Likewise, Safdar *et al.* (2012) also found a gradual increase in total soluble solids content of mango jam throughout the storage period of 150 days. Haq and Darakshan (2014) reported the gradual increase in the TSS during 60 days of storage period from 68 ºB (day 1) to 74 ºB (day 60). Similar results were reported by Ehsan *et al.* (2003) and Shakir *et al.* (2008). Touati *et al.* (2014) recorded the TSS of 64.42 ºB in apricot jam and after 60 days of storage time it increased up to ranged between 67.30 per cent. Kumar and Deen (2017) have reported the TSS of wood apple jelly in the storage duration of six months. They observed the slight increase in the TSS of jelly in the interval of one month up to six months. They found the TSS of 65.00, 65.15, 65.30, 65.65, 65.98, 66.30, 66.60 ºB in 0,1,2,3,4,5,6 months, respectively. Rahman *et al.* (2018) discussed about the significant increase in the TSS of guava jam from 67.4 to 69.71 ºB in the storage period of 90 days.

2.6.4 Ascorbic Acid

Mazur *et al*. (2014) observed a significant decrease of ascorbic acid concentration during storage (6 months at 20 °C) of strawberry jam. Djaoudene and Louaileche (2016) observed the decrease in the ascorbic acid content after storage the concentration of this compound decreased by 23.5 and 29 per cent, at 25 and 35 °C, respectively. Kumar and Deen (2017) also recorded the decrease in ascorbic acid in the wood apple jelly during 6 months storage period from 0.40 mg/100ml to 0.28 mg/100ml. Rahman *et al.* (2018) have determined the ascorbic acid content of the guava jam samples at 15 days interval for a period of 90 days. They recorded a slight decrease in the ascorbic acid content from 92.80 mg/100g and 71.52 mg/100g during the storage period of 90 days. In addition, Sutwal *et al.* (2019) reported that the ascorbic acid content in low calorie apple jam decreased from initial value of 13.99 to 13.90 mg/100g after 28 days.

2.6.5 pH

Muhammad *et al.* (2008) analyzed pH of different samples of apple jam during three months of storage period. The mean pH values decreased in all the samples from 4.60 to 3.72, 3.09 and 2.72 during initial, 30, 60 and 90 days of storage intervals. Whereas, Rababah *et al.* (2011) reported that strawberry jam had a significant decrease in pH in the storage period of 15 days. They found decrease from the initial pH value of 3.50 to 2.70 after 15 days of storage (25-55 ºC). Istrati *et al.* (2013) have reported the storage duration of ten days was not enough to cause significant decreases in pH i.e. in case of goji fruit jam pH decreased from 4.32 to 4.25 and pH of goji fruit jelly was reduced from 5.92 to 5.81. Touati *et al.* (2014) reported the significant decrease in the pH of apricot jam after prolonged storage where the initial values of pH (3.54) decreased to 3.39, 3.34 and 3.21 under temperature storage of 5 ºC, 25 ºC and 37 ºC, respectively. Similarly, in another study, pH of all the samples of apple-olive blended jam was reduced during the total storage period of 90 days. Decreasing trend in pH was reported due to the hydrolysis of pectic bodies and formation of acidic compound during degradation of sugar contents (Shah *et al.*, 2015).

2.6.6 Phenolic Contents

Rababah *et al.* (2011) observed the total phenols in the strawberry jam before storage was 848.86 mg GAE/100 g and after 15 days of storage at 25 °C the phenolic component of strawberry jam was recorded as 408.62 mg GAE/100 g. Kanwal *et al.* (2017) have recorded the mean values of total phenolic content of guava jam decreased from 78.92 mg GAE/100 g to 59.66 mg GAE/100 g during the 60 days of storage. Vukoja *et al.* (2019) have reported the total phenolic content in three different formulated cherry jams, after preparation of first jam phenolic content was 3.34 g/kg, while in the second sample and third sample had 2.29 g/kg and 1.99 g/kg, respectively. After 8 months of storage at room temperature, the highest retention of total phenolic content (85 %) was recorded in second and third sample.

2.6.7 Reducing Sugars

Kumar and Manimegalai (2005) studied the storage stability of whey (10 %) blended papaya (10 %) RTS beverage stored at refrigerated temperature for three months. During the storage period, a periodic increase in reducing sugars was observed, whereas a decrease in total sugars was reported. Istrati *et al.* (2013) observed the reducing sugars in the goji fruit jam and jelly in the storage period of 10 days. They have recorded the gradual increase in the reducing sugar in both the products. Haq and Darakshan (2014) observed the gradual increase in reducing sugar from 39.9 to 44.2 per cent in the apricot-date jam during the storage period of sixty days. Kanwal *et al.* (2017) have observed non-significant effect of different storage intervals on reducing sugars of guava jam. The mean values of reducing sugars for storage periods increased from 50.89 to 51.15 at 0 and 60 days, respectively.

2.6.8 Total Sugars

Sharma *et al*. (2008) observed a gradual and significant increase in total sugars in RTS beverages prepared from the blended guava and papaya juice during the storage from 13.71 per cent (initial) to 14.95 per cent (4 months of storage). Chauhan *et al.* (2012) indicated that the total sugar content of the coconut jam decreased by 0.56 per cent and 0.87 per cent after storage of 6 months at 28 ºC and 37 ºC, respectively. Whereas, Haq and Darakshan (2014) recorded a gradual increase in total sugars from 56.6 to 57.11 per cent in apricot-date jam during storage period of 60 days. Touati *et al.* (2014) reported that initial total sugars content of apricot jam decreased by 5.52 per cent, 9.02 per cent and 7.46 per cent after 60 days of storage at 5 ºC, 25 ºC, and 37 ºC, respectively. While, Kanwal *et al.* (2017) mentioned the increasing trend in total sugars of different guava jam samples during storage period of 60 days. They observed that the mean values of total sugars in storage (60 days) period increased from 63.39 to 63.56 per cent.

2.6.9 Sensory Properties

Sensory quality attributes of processed products have been affected during storage due to various metabolic changes. Chauhan *et al.* (2012) have reported that the sensory attributes such as colour, appearance, flavour and overall acceptability of the coconut jam samples showed a decreasing trend, while the spreadability remained almost constant throughout the storage period. Touati *et al.* (2014) have evaluated the sensory profile of the apricot jam in terms of colour, aroma, taste, spreadability and overall acceptability. They reported that interaction time–temperature factor had non- significant effect on the sensory profile of jam after storage, except for both spreadability and overall acceptability at 5 ºC. Palve *et al.* (2015) studied about the organoleptic properties of *Aloe vera* gel fortified pineapple jelly. They found the acceptable sensory score even after six months of the storage under ambient conditions of 20-30 ºC.

Colour is one of the most important parameters to the consumer while selecting the foods. Garcia-Viguera *et al.* (1999) also reported that the degradation and loss of red color in strawberry jam could be due to maillard and non-enzymatic browning, ascorbic acid degradation and polymerisation of anthocyanins with other phenolics during the thirty days of storage. According to Rababah *et al.* (2011) the redness values were the highest in the strawberry fruit (8.10) and decreased significantly after jam processing (5.65) and no significant difference during storage and among storage temperatures was observed. Touati *et al.* (2014) observed the reduction in the particular colour, lightness and changing from initial yellow to reddish tones in the apricot jam during the storage period of 60 days.

2.6.10 Microbiological Stability

Sharma (2014) recorded no microbial load up to four months in jamun mango blended jam, but after six months of storage a microbial count of (1×10^{-6} cfu per mL) was observed in first treatment (55:0, jamun-mango) and another treatment (35:20, jamun-mango) which falls in acceptable zone (Sharma, 2014). Whereas, Hamid *et al.* (2014) also found that 40 per cent fortification of *Aloe vera* gel resulted in zero yeast and mold count in orange-carrot nectars during 3 months of storage at ambient and at refrigerated temperature. In another study, Abedayo and Salam (2017) have also reported total microbial counts of $1\text{x}10^{3}$ cfu/ml in all the samples of spreads after 15-months storage. The samples were packed in the glass jars and found no fungal growth in the spreads. Isah (2017) reported very low microbial growth at the end of the 6 months storage in the locust bean jam whereas few counts were recorded for the locust bean syrup. Brandao *et al.* (2018) have reported the microbiological quality of dietetic functional mixed fruit jam during storage period of 180 days. They found that the mixed jam presented satisfactory sanitary standards of 10^{6} cfu/g even after 180 days of storage at ambient temperature (25-30 ºC). Makanjuola *et al.* (2019) have reported the total plate count in the date-orange-apple jam and date-apple jam was 55.00×10^{4} cfu/g and 17.67×10^{4} cfu/g, respectively.

2.7 APPLICATIONS OF RESPONSE SURFACE METHODOLOGY (RSM) FOR THE OPTIMIZATION OF PRODUCT FORMULATIONS

Response Surface Methodology (RSM) is a technique of optimization that can identify interrelationship between variables as being adopted by experiment/research studies in food preservation involving extraction process, fermentation as well as other discipline of engineering (Huang *et al.,* 2008; Olmez and Akbas, 2009). This approach develops a suitable experimental design that integrates all of the independent variables and uses the data input from the experiment to finally come up with a set of equations that can give theoretical value of an output (Said and Amin, 2015). In addition, RSM is also able to evaluate a single variable or the cumulative effect of the variables to the response. Although, this ability is shared with the other types of experimental design such as full factorial and partial factorial method, it differs in a way that the experimental runs are reduced (Wang *et al.,* 2012).

Ritthiruangdej *et al.* (2011) have reported the use of response surface methodology technique for the optimization of jack fruit sauce preparations. They developed a design with three independent variables as jackfruit puree (X_1; 45–55 %), sugar (X_2; 30–40 %) and vinegar (X_3; 5–15 %) on the chemical, physical and sensory qualities of jackfruit sauce were investigated. They found the best formulation of jackfruit sauce consisted of 45.9 per cent jackfruit puree, 32.4 per cent sugar, 11.7 per cent vinegar and developed jackfruit sauce with total soluble solids (38.57 °B), pH (3.72), moisture content (62.13 %), titratable acidity (0.07 %) and reducing sugar (4.13 %). Jain *et al.* (2016) have used the response surface methodology for optimizing the recipe for development of aonla squash with juice content (25 to 40%), acidity (1.00 to 1.10 %) as independent variables and adjusting 50 ºB total soluble solids. They observed that the RSM evaluated responses of 13 squash recipes by second order quadratic equations and analyzed that 40 per cent juice and 1.0 per cent acidity got highest score.

Heena *et al.* (2017) optimized the process for increasing the phyto-chemical, quality and nutritional attributes of spiced cucumber juice by using RSM. The blended cucumber juice with a 30.14 per cent sugarcane juice, 1 per cent herbal extract and a 1.5 per cent salt concentration was the most effective blend with the highest acceptability, phytochemical and quality attributes. Whereas, Kaur *et al.* (2018) has reported the best formulation of radish juice with 30 per cent sugarcane juice concentration, 1.0 per cent herbal extract and 1.5 per cent salt concentration was the most effective blend with the highest acceptability, phytochemical and quality attributes given by RSM. In another study, Kumari and Khatkar (2018) have optimized the best formulation of aonla jam by using response surface methodology as aonla pulp (95.92 g), sugar (66.20 g) and citric acid (0.5 g) and concluded that the F-values for models of gel strength, rupture force, adhesiveness and overall acceptability (209.29, 49.85, 55.58 and 76.18, respectively) was observed to be highly significant while the lack of fit was non-significant for all models event at 95 per cent level. Whereas, Mateen and Phanikumar (2018) have used the RSM design for the optimization of ber based kiwi fruit jam formulations as kiwi fruit pulp (90-100g/100g), ber pulp (5-15g/100g) and sugar (50-65g/100g).

Jayabalan and Karthikeyan (2013) reported that the response surface methodology (RSM) was used to optimize the variables like: *Aloe vera* juice (800-1200 mL), sugar (800-1200 g/kg), pectin (35- 60 g/kg) and citric acid (20-40 mL). They recorded that the best acceptability score for the *Aloe vera* jam formulations consist of *Aloe vera* juice 990 mL, sugar 1022 g/kg, pectin 50.3 g/kg and citric acid 28.2 mL. However, Sangma *et al.* (2018) developed sugarcane beverage by using statistical design (RSM), where the herb extract concentration varied from 0.5 per cent to 2.5 per cent, sugarcane juice concentration from 20 per cent to 50 per cent, and salt concentration from 0 per cent to 1.5 per cent. They concluded that the blend with 35.14 sugarcane juice concentration, 1 per cent herb extract, and 1.5 per cent salt concentration was best accepted and had the best quality attributes.

From the above review, it is clear that apple and *Aloe vera* based food products contain sufficient amount of functional compounds which are necessary for maintaining the health status and well being of consumers. Further, very less work has been cited in the literature on the production of fruit spreads using low calorie sweeteners. Hence, keeping above facts in view, the present research work was conducted to explore the possibility of developing *Aloe vera* fortified low calorie functional apple spread.

Chapter-3

MATERIALS AND METHODS

The present investigation entitled, "**Studies on development of *Aloe vera* fortified low calorie functional apple spread"** was carried out in the Department of Food Science and Technology, Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (HP) during the year 2018-2020. Different experiments were performed and evaluated in a sequence to achieve the objectives of the study. The details of the experimental material used and techniques adopted in the investigation are presented in this chapter under the following heads:

3.1 Materials
3.2 Experimental
3.3 Analyses

3.1 MATERIALS

3.1.1 Procurement of Raw Material

Fresh and mature apple fruits (*Malus* × *domestica* Borkh. var. Golden Delicious) were procured from local market, Solan (HP) and brought immediately to the Fruit Processing Laboratory of the Department of Food Science and Technology for further experimental studies. Whereas, the green and mature *Aloe vera* leaves were procured from the Department of Forest Products, Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (HP) and were also brought to the Department of Food Science and Technology for further studies.

3.1.2 Low Calorie Sweetener and Other Materials

The low calorie sweetener, '*Sweetos'* (Fructo-oligosaccharides+ Sucralose) was purchased from M/s Devendra Cottage Industry, Sector 20-C Chandigarh, India. Whereas, other materials like sugar, additives and packaging material were purchased from the local market, Solan (HP).

3.2 EXPERIMENTAL

3.2.1 Experiment 1: Extraction and Preservation of Pulp/Gel

3.2.1.1 Apple pulp

Apple fruits were weighed, sorted and washed thoroughly with cold water. Coring was done manually with the help of knife. Pulp was extracted by using hot pulping method, in which apple fruits were cooked in the open pan with 10 per cent water for 5-10 minutes followed by passing through a pulper. The extracted pulp was then homogenized, heated to boil and heat preserved in the glass bottles (90 ± 2 ^{o}C for 25 minutes). The bottles were kept at low temperature (7-10 ^{o}C) for further use in product development (Lal *et al.,* 2015). Pictorial illustration of unit operations used for the extraction and preservation of apple pulp is given in Plate 3.1 (a).

3.2.1.2 *Aloe vera* gel

The extraction of *Aloe vera* gel was done by using cold extraction method as described by Ramachandran and Rao (2008). Fresh and green leaves of *Aloe vera* were weighed and washed thoroughly with cold water. Then the lower leaf base of *Aloe vera*, the tapering point (2-4 inch) of the leaf top and the short, sharp spines located along the leaf margins were removed by a sharp knife and gel was scrapped with the help of a knife. The extracted gel was then passed through the fruit pulper to extract the uniform pulp. This pulp was boiled at 80 ºC, followed by pH adjustment (4.5) by adding citric acid to lower the pH and to improve its flow properties. The processed gel/pulp was filled in pre-sterilized glass bottles and processed at 90 ± 2°C for 25 min, followed by storage of bottles (Plate 3.1 b) at low temperature (7-10 ºC) for later use in product development (Chandegara and Varshney, 2013).

3.2.2 Experiment 2: Optimization of different process parameters (*Aloe vera* pulp, TSS, acidity and pectin) for the development of *Aloe vera* fortified apple spread

Different combination of the pulps (*Aloe vera* and apple) along with other variables i.e. total soluble solids (TSS), acidity and pectin were obtained according to the experimental Central Composite Design (CCD) (Table 3.1). The concentration of *Aloe vera* was varied between (10-40 %) per cent, total soluble solids (TSS) from 45 °B to 65 °B, acidity from 0.25 per cent to 1.25 per cent and pectin from 0.25 per cent to 1.25 per cent.

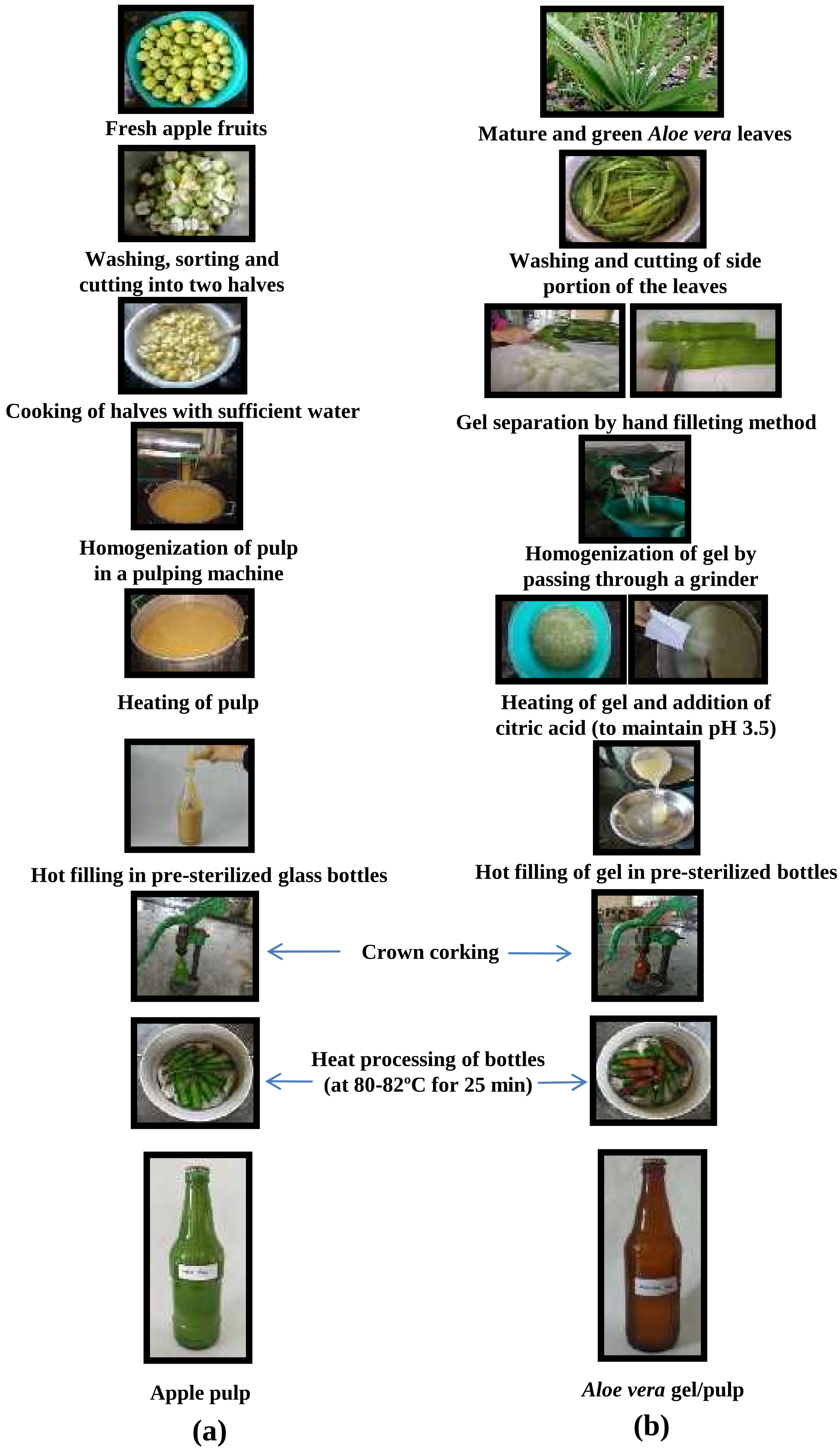


Plate 3.1 a) Pictorial depiction of process for extraction of apple pulp
b) Pictorial depiction of process for extraction of *Aloe vera* gel

Table 3.1: Ranges of the values for the RSM

Independent variables	Coded values				
	-α (-1.41)	**-1**	**0**	**+1**	**+α (1.41)**
A = *Aloe vera* pulp (%)	10	20	30	40	50
B = TSS (ºB)	45	50	55	60	65
C = Acidity (%)	0.25	0.5	0.75	1	1.25
D = Pectin (%)	0.25	0.5	0.75	1	1.25

Table 3.2: Details of the treatments for the preparation of *Aloe vera* fortified apple spread as designed by RSM

Runs/treatments	A= *Aloe vera* (%)	B= TSS (ºB)	C= Acidity (%)	D= Pectin (%)
1	20	50	0.50	1.00
2	30	65	0.75	0.75
3	30	55	0.75	0.75
4	20	50	1.00	1.00
5	40	50	0.50	1.00
6	20	50	1.00	0.50
7	20	60	1.00	1.00
8	20	60	0.50	0.50
9	20	60	0.50	1.00
10	20	50	0.50	0.50
11	30	55	1.25	0.75
12	30	55	0.75	0.75
13	30	55	0.75	0.75
14	40	50	0.50	0.50
15	30	55	0.75	1.25
16	30	55	0.75	0.75
17	40	60	0.50	1.00
18	20	60	1.00	0.50
19	40	60	1.00	1.00
20	30	55	0.75	0.25
21	30	55	0.75	0.75
22	10	55	0.75	0.75
23	30	45	0.75	0.75
24	30	55	0.75	0.75
25	50	55	0.75	0.75
26	40	60	0.50	0.50
27	40	60	1.00	0.50
28	40	50	1.00	0.50
29	30	55	0.25	0.75
30	40	50	1.00	1.00

Number of treatments: = 30
Design = RSM (CCD)

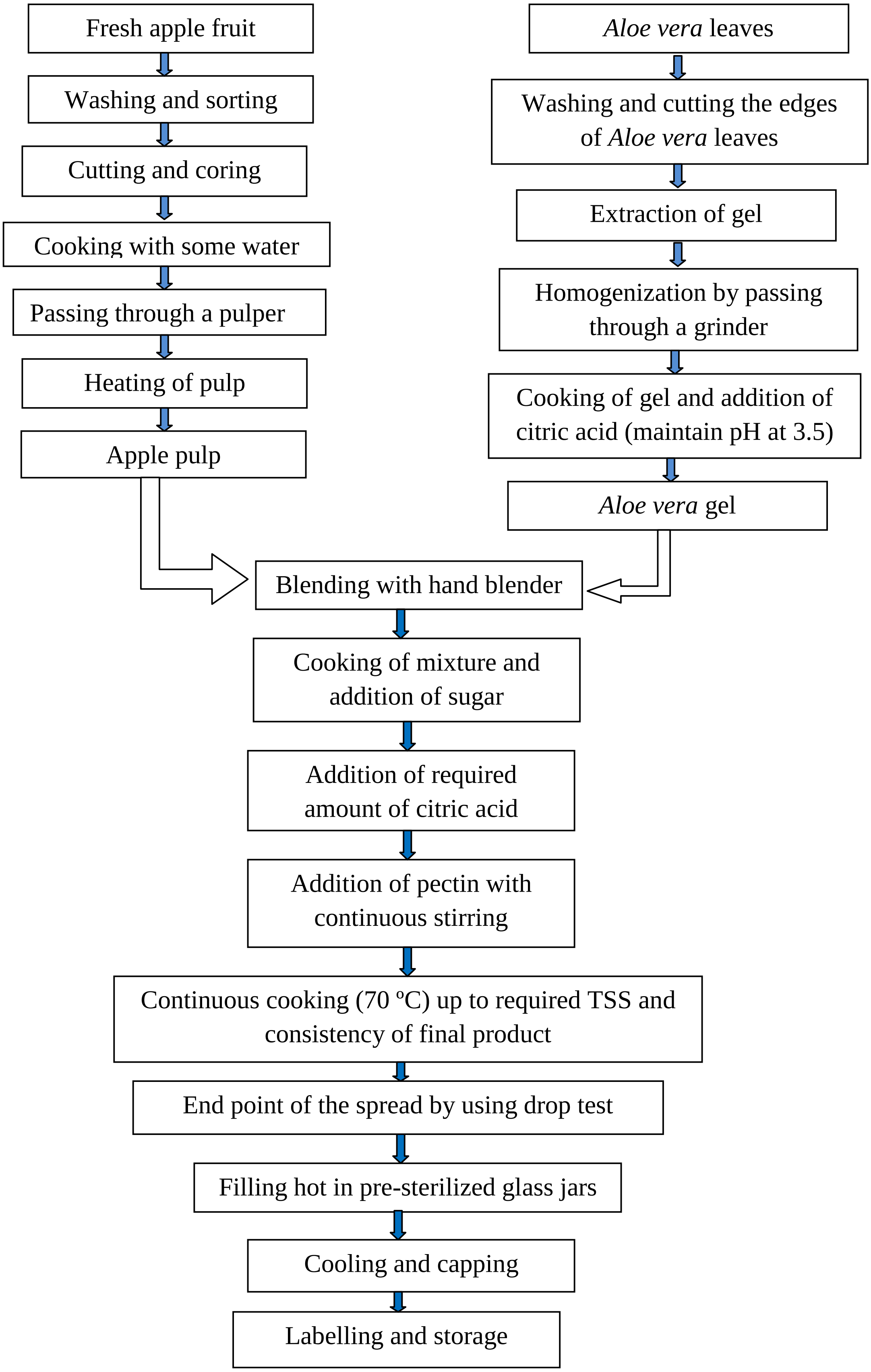
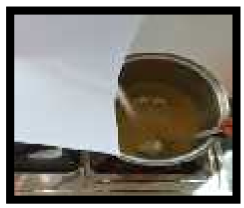


Figure 3.1 : Unit operation for the preparation of *Aloe vera* fortified apple spreads

Weighing of apple pulp and *Aloe vera* pulp

Homogenization with hand blender

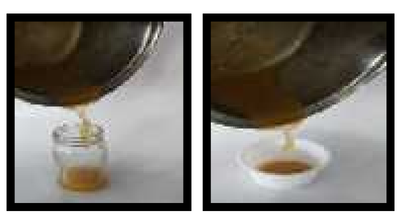
Cooking of mixture and addition of sugar



Addition of required amount of citric acid

Addition of required amount of pectin with continuous stirring

Continuous cooking (70 ºC) and measuring TSS



Filling hot in pre-sterilized glass jars and polypropylene cups

Cooling, labelling and storage at ambient conditions

Plate 3.2 Pictorial depiction of process for preparation of *Aloe vers* fortified apple spreads

The final volume was made up to 100 per cent using apple pulp in order to study the effect of multiple replicates of all variables on the physico-chemical, nutritional and sensorial characteristics of *Aloe vera* fortified functional apple spread. Response Surface Methodology (RSM) was used to predict the optimum concentration (percentage) of *Aloe vera* (A), TSS (B), acidity (C) and pectin (D). To investigate the linear and quadratic effect of all the variables and their interactions on dependent variables, second – order experimental design i.e., CCD with four factors of five levels (-α, -1, 0, +1, +α) was employed. The design involved 6 centre design points with α value being ± 1.41 with total number of 30 runs. The coded levels of responses as -1, 0 and +1 were investigated in the current study. The ranges of the coded values of RSM and the details for experimental design are depicted in Table 3.1 and 3.2, respectively. The best treatment was selected on the basis of different responses for further studies. The spread was prepared by using standard method given in the literature (Sorour *et al.,* 2016) as shown in the Plate 3.2. Fruit spread is a jam like product which was prepared by cooking fruit pulp along with sugar, citric acid and pectin. It was cooked until the gel like consistency achieved and hot filled in a glass jar for preservation. Unit operations used for the preparation of *Aloe vera* fortified apple spread are depicted in Figure 3.1.

3.2.3 Experiment 3: Optimization of different concentrations of sweetos (FOS-sucralose) for the development of *Aloe vera* fortified low calorie functional apple spread

Before the preparation of *Aloe vera* fortified low calorie apple spread, the relative sweetness of sweetos was calculated by using Duo-trio sensory evaluation test (Appendix-I) as shown in Table 3.3. So, on the basis of equivalent sweetness, the quantity of sweetos was used to replace sucrose in the product.

Table 3.3: Relative sweetness of non-nutritive sweetener used

Sweetener	Intensity of sweetness (viz-a-vis sucrose = 1)
Sweetos	Equivalent to sucrose (Table sugar)

The overall best treatment from Experiment- 2 was used for the development *Aloe vera* fortified low calorie functional apple spread by replacing sugar (sucrose) sweetness with equi-sweetness of sweetos at different proportions as given in the Table-3.4. The concentrations of pectin was varied from 0.5 to 1 per cent, whereas the acidity was kept constant (0.5) in all the treatments. The *Aloe vera* fortified low calorie functional apple spreads were prepared by boiling calculated concentrations of sugar and sweetos and mixed with the pulp. Whereas, recommended bulking agent i.e. pectin was added by mixing with the small quantity of sweetos/sugar as per the detail of treatments (Table 3.4). The mixture was cooked at 70 ºC until the desirable consistency achieved (Sorour *et al.,* 2016). The spreads were then hot filled in the pre-sterilized glass jars (200 mL capacity) and polypropylene cups (200 mL capacity) separately and were stored at ambient temperature along with control sample to study their storage stability for a period of 6 months.

Table 3.4: Details of treatment for the development of *Aloe vera* fortified low calorie functional apple spread

Treatments	% Equivalent sweetness used (sucrose=1)		Bulking agent (%)
	Sucrose	Sweetos	Pectin
T_1	100	-	-
T_2	75	25	0.5
T_3	75	25	1.0
T_4	75	25	1.5
T_5	50	50	0.5
T_6	50	50	1.0
T_7	50	50	1.5
T_8	25	75	0.5
T_9	25	75	1.0
T_{10}	25	75	1.5
T_{11}	0	100	0.5
T_{12}	0	100	1.0
T_{13}	0	100	1.5

Number of treatments = 13
Replication = 3
Design = RBD/CRD, as applicable.

3.2.4 Experiment 4: Storage stability of *Aloe vera* fortified low calorie functional apple spread

The apple spreads from Experiment 2 and 3 were analyzed for various physico-chemical, nutritional, microbiological and sensory characteristics at different intervals of 0, 3 and 6 months during storage at ambient temperature (Appendix-II).

Number of treatments	= 3 (Apple spread, *Aloe vera* fortified apple spread and low calorie apple spread)
Packaging material	= 2 (glass jars and polypropylene cups)
Storage period	= 3 (0, 3 and 6 months)
Total number of treatments (3x2x3)	= 18
Replications	= 3
Design	= CRD factorial/RBD as applicable.

3.3 ANALYSES

Fresh apple fruit, *Aloe vera* leaves and the developed products were analyzed for various physico-chemical, microbiological and sensory characteristics (as applicable) *viz.* weight, edible portion, pulp yield, visual color, spreadability, cooking time, water activity, moisture content, total phenols, total soluble solids (TSS), total sugars, reducing sugars, titratable acidity, pH, ascorbic acidity, ash content, antioxidant activity, fructo-oligosaccharide (FOS) content, energy value microbial examination and sensory attributes as per standard methods described below under different heads:

3.3.1 Physical Characteristics

The randomly selected 10 number of each apple fruit and *Aloe vera* leaves were analyzed for the following characteristics.

3.3.1.1 Weight

The weight of the randomly selected samples of apple and *Aloe vera* leaves was measured with the help of digital weighing balance and an average weight was calculated and expressed in grams (g).

3.3.1.2 Size

The size of fruits and *Aloe vera* leaves was determined by measuring the length and diameter with the help of digital vernier calliper. The average size (length and diameter) was expressed in centimeters (cm).

3.3.1.3 Volume

Volume of apple fruit was measured by water displacement method. Ten randomly selected samples were dipped in a measuring cylinder containing water. The amount of water displaced or increase in level of water in measuring cylinder was recorded and expressed as volume (cc) of fruit.

3.3.1.4 Edible portion

The weight of edible portion (containing only pulp/gel) and whole fruit/leaf was measured separately with the help of digital weighing balance. The per cent edible portion was calculated by using the given formula:

$$\text{Edible portion (\%)} = \frac{\text{Weight of edible portion (g)}}{\text{Weight of whole fruit (g)}} \times 100$$

3.3.1.5 Pulp and gel yield

Pulp of apple fruit was extracted by hot pulping extraction method, whereas *Aloe vera* gel was extracted by cold extraction method. The total yield of pulp/gel was expressed in percentage and calculated by using the following formula:

$$\text{Pulp yield (\%)} = \frac{\text{Weight of gel/pulp extracted (g)}}{\text{Weight of sample taken (g)}} \times 100$$

3.3.1.6 Visual colour

The colour of selected samples was observed visually by comparing with the colour cards of Royal Horticulture Society, London.

3.3.1.7 Spreadability

Spreadability was analyzed by placing the pre weighed sample in circular glass plate or petri dish followed by placing a known weight over it so as to enable the sample to spread. After one minute, the area (diameter) covered by the sample in opposing directions (spread) was noted with a scale as per the procedure described by Deuschle *et al.* (2015). The average diameter was recorded and spreadability factor was calculated by using the following equation:

$$\text{Spreadability factor (cm}^2\text{/g/min)} = \frac{\text{Total area (cm}^2\text{)}}{\text{Total weight (g)}}$$

3.3.1.8 Cooking time

The total time required by the apple spreads for cooking to desired consistency was observed with the help of stopwatch.

3.3.2 Chemical Characteristics

3.3.2.1 Water activity

Water activity was analyzed by computer digital water activity meter (HW_3 model, Rotronic International, Switzerland), where direct measurements were taken at room temperature. Standard cuvetts were used in which products were filled up to the rim with negligible air gaps and placed below the sensor of the water activity meter. It gave direct reading of water activity of the sample at room temperature.

3.3.2.2 Moisture content

Moisture was estimated by drying the weighed sample to a constant weight in a hot air oven (AOAC, 2004). Ten gram of sample was weighed in petri dish with lid and was placed in hot air oven at 65 ± 2 ºC and dried until constant weight was achieved. The per cent moisture content was calculated as follows:

$$\text{Moisture content (\%)} = \frac{\text{Weight of fresh sample (g)} - \text{Weight of dried sample (g)}}{\text{Weight of fresh sample (g)}} \times 100$$

3.3.2.3 Total phenols

The amounts of total phenols in the sample were determined by using the method given by AOAC (2012) with the Folin-Ciocalteu reagent using gallic acid as a standard. One g of sample was taken and ground with 10 mL 80 per cent ethanol in pestle and mortar and centrifuged for 20 min at 1000 rpm and filtered. The Filtrate was evaporated in oven up to dryness and residue was dissolved in 5 mL of distilled water. An aliquot of 0.2 to 2 mL was taken in separate test tubes and volume was made up to 3 mL with water. Then 0.5 mL Folin-Ciocalteu reagent was added. After 3 min, 2 mL of Na_2CO_3 (20 %) was added and mixed. Test tubes were placed in boiling water bath for one min and then cooled. Optical density of the sample was recorded at 650 nm with the help of UV-vis spectrophotometer (Shimadzu, Japan). The concentration was determined as per the standard procedure from the standard curve (Appendix-III). The standard curve was prepared using different concentrations (8-32 µg/ mL) of gallic acid and results were expressed as mg per 100 g on fresh weight basis.

3.3.2.4 Total soluble solids

The TSS of ripe apple fruit, *Aloe vera* gel and fruit spread was determined with the help of hand refractometer of three different calibrations i.e. 0-32, 28-62 and 58-92 ºB. The samples were prepared by following the procedure given in AOAC (2004).

3.3.2.5 Sugars

Sugars were estimated by Lane and Eynon volumetric method as explained by Ranganna (2009). The sample was prepared and neutralized with 1N NaOH, using phenolphthalein as an indicator. To this, 2 mL of neutralized lead acetate was added and the solution was kept standing for 10 minutes. After that, necessary amount of potassium oxalate solution was added to precipitate excess of lead then made up to the volume and filtered. The filtrate was used for estimation of reducing and total sugars.

a) Reducing Sugars: The filtered sample was taken in 50 mL burette and titrated against 10 mL mixed Fehling A and B solution using methylene blue as an indicator. The end point was indicated by the appearance of brick red colour. The reducing sugars were calculated as:

$$\text{Reducing sugars (\%)} = \frac{\text{Factor} \times \text{Dilution}}{\text{Titre value} \times \text{Weight or volume of sample}} \times 100$$

b) Total sugars: Clarified filtrate (50 mL) was taken into 250 mL conical flask and 5 g citric acid and 50 mL water was added to it. The mixture was boiled gently for ten minutes and then cooled. The solution was neutralized with 1N NaOH, using phenolphthalein indicator and volume was made up to 250 mL. Total sugars were determined in a same way as reducing sugars.

Total sugars (%)	=	Calculated as in reducing sugars making use of titre value obtained in the determination of total sugars after inversion

3.3.2.6 pH

The pH in apple fruit pulp, *Aloe vera* gel and spread was determined by using a digital pH meter (CRISON Instrument, Ltd. Spain). The sample was finely crushed and diluted with distilled water to make an extract. Before estimating the pH of sample, pH meter was calibrated with standard buffers of 4, 7 and 9 pH as per standard procedure (Ranganna, 2009).

3.3.2.7 Titratable acidity

The titratable acidity was determined by titrating a known volume of the sample against 0.1N NaOH solution by using phenolphthalein as an indicator (AOAC, 2004). The per cent titratable acidity was calculated by using the following formula:

$$\text{Titratable acidity (\%)} = \frac{\text{Titre value} \times \text{Normality of alkali} \times \text{Volume made up} \times \text{Eq.wt. of acid}}{\text{Weight of sample taken} \times \text{Aliquot taken for estimation} \times 1000} \times 100$$

3.3.2.8 Protein

The crude protein content was estimated as per method described by AOAC (2012) by using semi-automatic instrument i.e. KjelTRON (KDIGB 6M &KjelDISTEA). A pre-weighed moisture free sample of approximately 0.20 g was digested in digestion tubes after adding 10 mL of concentrated sulphuric acid and 3.00 g of digestion/catalyst mixture (potassium sulphate and copper sulphate in 5:1 ratio) at 400 ºC in the digestion unit. Left the tubes for one hour and the appearance of clear bluish green color liquid indicated the completion of digestion. After cooling, an aliquot was transferred to the automatic distillation unit where aliquot was diluted, made alkaline by mixing with 40 per cent NaOH solution and was distilled. Liberated ammonia was collected in a conical flask containing 25 mL of 4 per cent boric acid solution and 2-3 drops of mixed indicator (Methyl red and bromocresol green). The distillate (Bluish green color) obtained was titrated against standard 0.1 N H_2SO_4 to light pink end point. The percentage crude protein was calculated by multiplying percent nitrogen by factor 6.25. A parallel blank was run to eliminate the error.

$$\text{Nitrogen (\%)} = \frac{14 \times \text{Normality of acid} \times \text{Titre value}}{\text{Weight of sample taken} \times 1000} \times 100$$

$$\text{Protein (\%)} = \text{\% of Nitrogen} \times 6.25$$

3.3.2.9 Fat

Fat content was determined by the method given by AOAC (2012) by using the automatic SoxTron fat extraction system (Model: Sox-2 version 0.1) instrument. Initially weight of the collection vessel was taken (Initial weight) and 5 g of the powdered sample was taken in thimbles and placed in thimble holder and keep the holder in a collection vessel and to this 80 mL petroleum ether was added. The fat extraction process was carried out for 45 min by setting the temperature at 90 ºC. After 45 min, the collection vessels were kept in an oven at 100 ºC for 10-15 min to evaporate the petroleum ether. The beakers were then cooled in desiccators and weighed again (Final weight). The fat content was calculated using the following formula:

$$\text{Crude Fat (\%)} = \frac{W_1\text{-}W_2}{W} \times 100$$

Where, W (g) is weight of the sample; W_1 (g) is weight of the empty beaker and W_2 (g) is the weight of the empty beaker + fat content (ether extract)

3.3.2.10 Non-enzymatic browning (NEB)

The non-enzymatic browning was recorded according to the procedure outlined by Ranganna (2009). The colour of sample was measured at 440 nm by UV-vis double beam spectrophotometer using 60 per cent aqueous alcohol as blank solution. The absorbance was expressed as browning in terms of O.D.

3.3.2.11 Ascorbic acid (Vitamin C)

The titrimetric method using 2, 6- dichlorophenol-indophenol dye was followed for the determination of ascorbic acid in different products. A known quantity of sample (raw fruit and fruit spread) was extracted in 3 per cent meta-phosphoric acid and titrated with dye to light pink colour persisting for at least 15 seconds. The dye was standardized by titrating against standard ascorbic solution (0.1 mg L-ascorbic acid per mL of 3 per cent HPO_3 solution) and dye factor was calculated by using following formula:

$$\text{Dye factor} = \frac{0.5}{\text{Titre value}}$$

$$\text{Ascorbic acid (mg/100 g)} = \frac{\text{Titre value} \times \text{Dye factor} \times \text{Volume made up}}{\text{Aliquot of extract taken for estimation} \times \text{Weight/Volume of sample taken for estimation}} \times 100$$

3.3.2.12 Total ash

The total ash content was determined gravimetrically (AOAC, 2004) by taking known weight of samples in tared silica crucibles. The dried samples after moisture determination were slowly heated over hot plate until the bulk of organic matter was burnt. The crucibles were then placed in a muffle furnace for ashing at 550 ^{o}C to obtain a carbon free white ash with a constant weight. Ash content of sample was then calculated and expressed as per cent on fresh weight basis.

$$\text{Ash (\%)} = \frac{\text{Weight of ash (g)}}{\text{Weight of sample (g)}} \times 100$$

3.3.2.13 Antioxidant activity

The antioxidant activity in bar was measured as per the method of Brand-Williams *et al*. (1995). DPPH (2, 2-diphenyl-l-picrylhydrazyl) was used as a source of free radical. A quantity of 3.9 mL of 610-5 mol/L DPPH in methanol was put in cuvette with 0.1 mL of sample extracted and decrease in absorbance was measured at 515 nm for 30 minutes or until the absorbance became steady. Methanol was used as blank. The remaining DPPH concentration was calculated using following equation:

$$\text{Antioxidant activity (\%)} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

3.3.2.14 Minerals

Samples were weighed and dried in the hot air oven (65 ± 5 ºC) for 48 hours. The dried samples were crushed, ground and stored in butter paper bags for the estimation of various minerals (Chapman, 1964). For the estimation of K, Ca, P, Fe, Cu, Mn and Mg, the spread and pulp samples were digested in di-acid mixture prepared by mixing HNO_3 and $HClO_4$ in the ratio of 4:1 taking all precaution as suggested by Piper (1966).

a) Calcium and Potassium

Pre digested samples were diluted with 75 mL distilled water and minerals were estimated by using flame photometer.

b) Phosphorus

Total phosphorus was estimated by Vanado Molybdate Phosphoric Yellow Colour Method (Jackson, 1973). Five mL of aliquot (digested) was pipette out in a 25 mL volumetric flask and 5 mL of vanado molybdate reagent was added. Then final volume was made up to 25 mL with distilled water and allowed to develop colour for half an hour. After the development of colour, concentration of phosphorus in the solution was recorded on UV-Spectrophotometer Spectronic 20D at 470 nm wavelength and a blank was run simultaneously to adjust zero absorbance. Phosphorus content was expressed in g/100mL.

c) Magnesium, iron, copper and manganese

Atomic absorption spectrophotometer was used for direct estimation of Mg, Fe, Cu and Mn in the pre digested spread samples.

3.3.2.15 Qualitative estimation of aloin (TLC)

The qualitative estimation of aloin was done with the help of Thin Layer Chromatography (TLC) which was done on Silica Gel-G coated aluminium plates (20cm×10cm, 0.25 mm thickness) samples were placed along with aloin standard with the help of hamilton syring. The aloin was separated by dipping sample containing plates in ethyl acetate: methanol: water (100:13.5:10) mobile phase. The separated spots and R_f values were recorded by spraying with 5 per cent ethanolic potassium hydroxide and saw in the ultra violet light chamber (Pandey *et al.,* 2016).

$$R_f = \frac{\text{Distance moved by solute from the origin}}{\text{Distance moved by solvent from the origin}}$$

3.3.2.16 Energy value (Kcal)

Energy value of developed products was analyzed with the help of bomb calorimeter (Model Toshiwal DT-100). The spread was first dried in as oven to remove the water content and converted into solid matter. For the estimation of energy value one gram of dried sample was required. Distilled water (1500 mL) was filled in the chamber (Dewar) and attached to one of the ignition wire and then, to the central terminal on the bomb head. Second wire was attached to the socket provided on the bomb hanger. A thread was tied with the ignition wire and pushed down into the sample. The air was replaced with oxygen and with the increase in temperature, sample ignited automatically in the assembly. After complete ignition of the sample the calorific value was shown on the display screen of instrument as Kcal/g of sample (Sharma *et al.*, 2020).

3.3.3 Sensory Evaluation

Nine point hedonic scale method given by Amerine *et al.* (1965) was followed for conducting the sensory evaluation of pumpkin fruit bar. The panel of ten judges comprising of faculty members and post graduate students of department of Food Science and Technology, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan (HP) were selected with care to evaluate the product. Efforts were made to keep the same panel for sensory evaluation throughout the entire period of study. The samples were presented to the judges the way they are normally consumed. Plain water was given to the judges to rinse their mouth in between the evaluation of samples. Coded samples were presented to the judges in separate chambers or places to get unbiased judgments. Each sample was evaluated for various sensory attributes *viz.* appearance, texture, spreadability, flavour and overall acceptability on 9-point Hedonic scale signifying: 9 = liked extremely and 1= disliked extremely (Appendix-IV).

3.3.4 Microbiological Examination

Total plate count was calculated by aseptically inoculating 0.1 g of serially diluted samples in total plate count/standard plate count agar medium prepared according to Ranganna (2009). One mL of sample after serial dilution (10^{-2}, 10^{-4}, 10^{-6} and 10^{-8}) was aseptically inoculated in pre sterilized plates, followed by pouring total plate count agar (10-15 mL) under sterilized environment of laminar air flow. The plates were then incubated at 37 ºC for 72 hrs prior counting of microbes. The results of total plate count (TPC) were expressed as x 10^{-4} cfu/g of sample.

3.3.5 Statistical Analysis

Statistical analysis of the quantitative data of chemical parameters obtained from the experiments was determined by Completely Randomized Design (CRD) factorial. Whereas, the data pertaining to sensory evaluation of the samples was analyzed by Randomized Block Design (RBD) as given by Mahony (1985) using one factor, two factor and three factor analysis of variance (ANOVA) with the help of OPSTAT. However, for the optimization of variables viz, *Aloe vera* gel (%), TSS (ºB), acidity (%) and pectin (%) in Experiment -2, the statistical analysis was carried out by using Expert version 7.00. Central Composite Design of Response Surface Methodology and multiple regressions ANOVA was used to analyze and predict the conditions using second order polynomial equations (regression equation) and three dimensional surface plots.

A second order response for 4 independent variables was expressed by the equation below:

$$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_4 + \beta_{12}X_1X_2 + \beta_{13}X_1X_2 + \beta_{14}X_1X_2 + \beta_{23}X_2X_3 + \beta_{24}X_2X_4 + \beta_{34}X_3X_4 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{33}X_3^2 + \beta_{44}X_4^2$$

where, Y is the response to be calculated

X_1, $X_{2,}$ X_3 and X_4 are coded value of independent variables

β_0 is model coefficient

β_1, β_2, β_3 and β_4 are linear regression coefficients

β_{11}, β_{22}, β_{33} and β_{44} are quadratic regression coefficients

β_{12}, β_{13}, β_{14},β_{23}, β_{24} and β_{34} are interactive regression coefficients

3.3.6 Cost of Production

The cost of raw material like fruits, *Aloe vera* leaves, sugar, non-nutritive sweeteners, packaging material, additives etc. were purchased as per current price in the market and the cost of final products were calculated taking into account the cost of raw material, ingredient, packaging material and processing cost.

Chapter-4

RESULTS AND DISCUSSION

The present scientific work entitled, **“Studies on the development of *Aloe vera* fortified low calorie functional apple spread”** was carried out in the Department of Food Science and Technology, Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (HP) during the year 2018-2020. The results of this study are presented and discussed in this chapter under different heads given below:

4.1 Physico-chemical characteristics of fresh apple fruits and *Aloe vera* leaves
4.2 Quality characteristics of fresh apple pulp and *Aloe vera* gel
4.3 Optimization of parameters for the development of *Aloe vera* fortified apple spread using Response Surface Methodology (RSM)
4.4 Optimization of concentrations of sweetos (FOS-sucralose) and pectin for the development of low calorie *Aloe vera* fortified apple spread
4.5 Physico-chemical and sensory characteristics of apple spread, *Aloe vera* fortified apple spread and low calorie apple spread
4.6 Changes in the quality attributes of apple spreads during storage
4.7 Microbial quality of apple spreads during storage
4.8 Cost of production of *Aloe vera* fortified apple spreads

4.1 PHYSICO-CHEMICAL CHARACTERISTICS OF FRESH APPLE FRUITS AND *ALOE VERA* LEAVES

The data pertaining to various physico-chemical characteristics of fresh apple fruit and *Aloe vera* leaves are presented in Table 4.1 and described below under suitable captions:

4.1.1 Apple

Apple (*Malus* × *demestica* Borkh), a member of sub family Pomoideae in Rosaceae family is one of the most important fruits of temperate regions of the world (Khan *et al.,* 2017). It is being used as fresh as well as in processed form because of its secondary metabolites including polyphenol, antioxidants, vitamins and minerals (Kumar *et al.,* 2018). The recent epidemiological studies indicated that apple consumption is associated with reduced risk of cardiovascular diseases, asthma, diabetes and some cancers (Kotiyal *et al.*, 2017). Although, many researchers extensively investigated the physico-chemical properties of different apple cultivars (Rana and Bhushan, 2016), but the apple fruits (Golden Delicious) used in the present investigation were also studied for physico-chemical characteristics. Perusal of data showed that the average length, diameter, weight and volume in apple fruits were recorded as 66.91 mm, 65.11 mm, 127.03 g and 169.16 cc, respectively which were in accordance to the physical characteristics obtained by Verma *et al.* (2018).

The visual colour of Golden Delicious was recorded to be yellow-green 145 group (145-B) by visual comparison with Royal Horticulture Colour Cards. The edible portion and pulp yield was observed as 88.5 per cent and 79.20 per cent, respectively. The pictorial depiction of apple pulp and *Aloe vera* gel was presented in the Plate 4.1. The average moisture content, total soluble solids, reducing sugar, total sugar, titratable acidity, pH and ascorbic acid content in apple fruits were recorded as 85.24 per cent, 13.13 ºB, 7.35 per cent, 12.39 per cent, 0.46 per cent, 3.44 and 16.7 mg/100g, respectively (Table 4.1). Whereas, Chakespari *et al.* (2010) and Yosef and Belal (2014) reported the total soluble solids ranged between 9.98 ºB to 11.22 ºB in apple fruit. While, Kishor *et al.* (2017) observed reducing sugars and total sugars in Golden Delicious fruit as 7.90 per cent and 9.58 per cent, respectively. Whereas, titratable acidity (0.39 %), pH (4.20) and ascorbic acid (10.42 mg/100g) were observed by Verma *et al.* (2018) in Golden Delicious apples. Further, data in the Table 4.1 also revealed that the total phenols and antioxidant potential were recorded as 66.14 mg/100g and 58.63 per cent, respectively which were almost similar to the results recorded by Jelodarian *et al.* (2012) and Bondonno *et al.* (2017) in Golden Delicious apple fruits.

4.1.2 *Aloe vera*

Aloe vera (syn. *Aloe barbadensis* Miller), a member of Asphodelaceae (Liliaceae) family is a spiky, succulent, perennial plant and a native to warm dry regions of the world. It is cultivated almost everywhere in the world, both as an indoor plant and for its medicinal qualities (Chandegara and Varshney, 2013). The *Aloe vera* leaves used in the present study (Table 4.1) had average length, diameter and weight as 265.44 mm, 79.71 mm, 244.93 g, respectively. While, according to Azam *et al.* (2014) and Pandey and Singh (2016) the range of leaf weight, length and diameter were found as 429 to 450 g, 300 to 321 mm and 90 to 123 mm, respectively.

The *Aloe vera* leaves used in the present study fell under Green 139 group (139-B) measured by Royal Horticulture Colour Cards. The average percentage of edible portion and pulp/gel yield were observed as 66.39 per cent and 48.59 per cent, respectively which was lower than the gel recovery reported by Chandegara and Varshney (2013). The average moisture content, total soluble solids, reducing sugars, total sugars, titratable acidity, pH and ascorbic acid was recorded as 98.27 per cent, 2.04 ºB, 0.61 per cent, 1.21 per cent, 0.22 per cent, 4.54 and 10.07 mg/100g, respectively. It might be due to variation in growing conditions and handling during processing of leaves. While, a slight variation was observed in moisture content and TSS of *Aloe vera* leaves which ranged between 95.0 to 98.5 per cent and 1.92 to 2.02 ºB, respectively (Chaisawadi *et al.,* 2005; Ahmed and Hussain 2013). Whereas, Burang (2018) obtained 0.58 per cent reducing sugars, 1.02 per cent total sugars, 0.25 per cent titratable acidity, 4.5 pH and 7.89 mg /100g ascorbic acid in *Aloe vera* leaves. Also, total phenols and antioxidants potential of *Aloe vera* leaves used in the present study were recorded as 217.53 mg/100g and 82.98 per cent, respectively. These findings were in accordance with the range reported by Abed *et al.* (2012) and Sharma *et al.* (2015).

Table 4.1 Physico-chemical characteristics of fresh apple fruit (Golden Delicious) and *Aloe vera* leaves

Parameters*	Mean ± SE	
	Apple fruits	*Aloe vera* leaves
Length (mm)	66.91 ± 0.97	265.44 ± 1.38
Diameter (mm)	65.11 ± 0.37	79.71 ± 1.37
Weight (g)	127.03 ± 0.84	244.93 ± 2.17
Volume (cc**)	169.16 ± 0.31	ND
Visual colour ***	Yellow-Green 145 group (145-B)	Green 139 group (139-B)
Edible portion (%)	88.5 ± 0.28	66.39 ± 2.31
Pulp yield (%)	79.20 ± 1.03	48.59 ± 0.30
Moisture (%)	85.24 ± 0.30	98.27 ± 0.35
Total Soluble Solid (ºB)	13.13 ± 0.20	2.04 ± 0.02
Titratable acidity (%)	0.46 ± 0.01	0.22 ± 0.02
pH	3.44 ± 0.10	4.54 ± 0.07
Reducing sugars (%)	7.35 ± 0.55	0.61 ± 0.01
Total sugars (%)	12.39 ± 0.26	1.21 ± 0.01
Ascorbic acid (mg/100g)	16.7 ± 0.25	10.07 ± 0.37
Total phenols (mg/100 g)	66.14 ± 0.18	217.53 ± 0.67
Antioxidant potential (% DPPH free radical scavenging activity)	58.63 ± 0.34	82.98 ± 0.25

***** Each physical parameter is average of 10 determinations, whereas the chemical parameters are the average of three determinations; SE = Standard Error; ND = Not Detected

**Volume was analyzed in cubic centimeter (cc)

***Visual colour value of apple and *Aloe vera* leaves was recorded by colour card of Royal Horticulture Society, London

4.2 QUALITY CHARACTERISTICS OF FRESH APPLE PULP AND *ALOE VERA* GEL

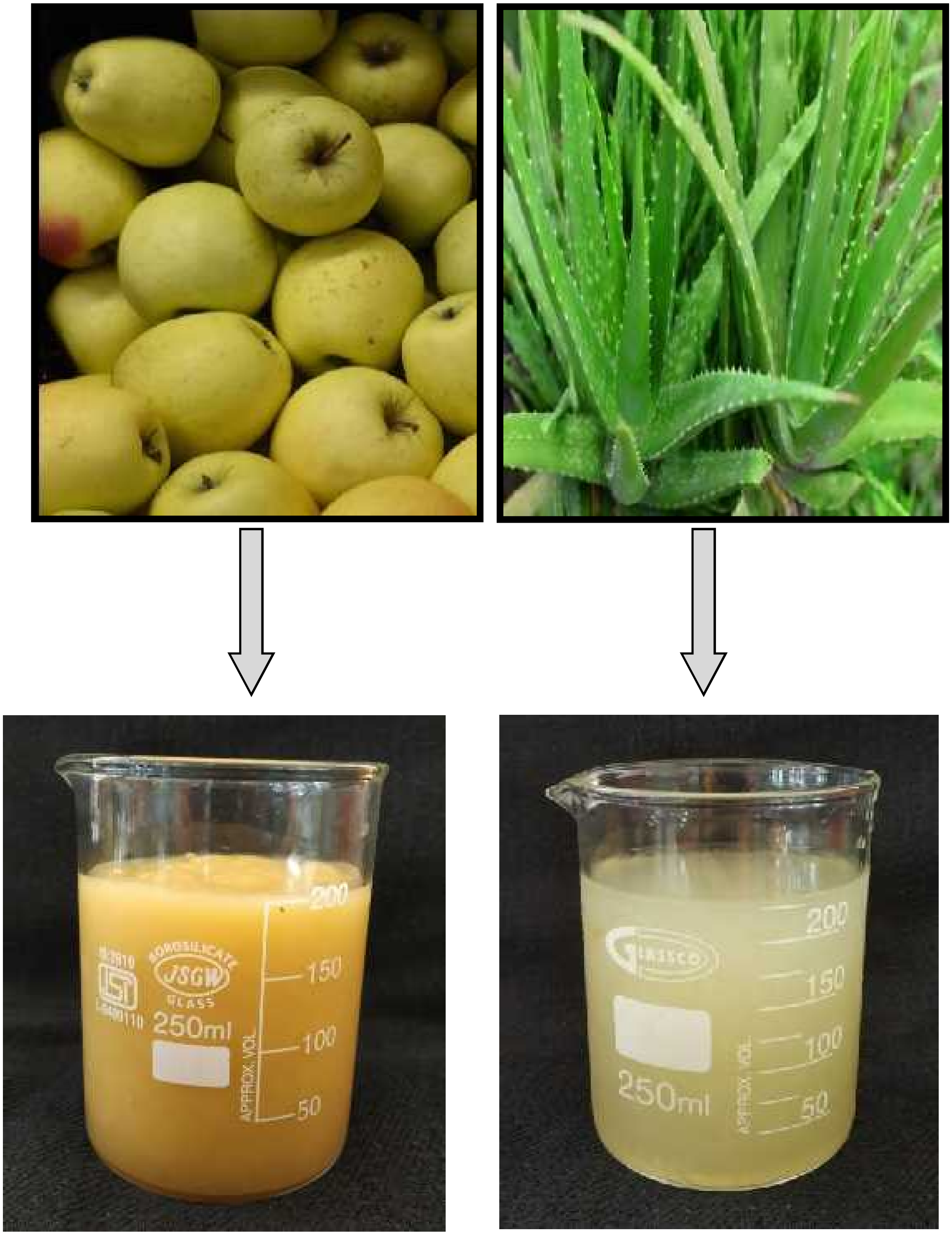
The data recorded for quality characteristics of fresh apple pulp and *Aloe vera* gel are presented in Table 4.2 and discussed below under suitable captions:

4.2.1 Apple Pulp

Apple pulp was extracted by using hot pulping method and filled in the pre sterilized glass bottles and analyzed for various physico-chemical attributes (Table 4.2). Similar extraction method was also discussed by Arampath and Dekker (2019) in the mango pulp. The pulp used in the present study was found to contain 13.62 ºB total soluble solids, 0.49 per cent titratable acidity, 3.39 pH, 7.48 per cent reducing sugars, 12.64 per cent total sugars and 10.50 mg/100g ascorbic acid. While, Muhammad *et al.* (2011) and Nisar *et al.* (2015) reported average titratable acidity (0.32 %), pH (3.59), total soluble solids (9.75 ºB) and ascorbic acid (18.96 mg/100g) of apple pulp. The values for total phenols (40.30 mg/100g) and antioxidant potential (34.48 per cent) recorded in apple pulp were almost similar to the results obtained by Manzoor *et al.* (2012) i.e. 43.12 mg/100g and 35.93 per cent, respectively. Apple pulp also found to contain minerals such as potassium (0.162 g/100g), phosphorus (0.480 g/100g) and calcium (40.00 mg/100g).

4.2.2 *Aloe vera* Gel

Aloe vera gel was extracted by hand filleting method and hot filled in the pre sterilized glass bottles after heating. Then bottles were processed for 15-20 minutes and preserved for further use (Hamid *et al.*, 2014). The preserved gel was analyzed for different quality characteristics (Table 4.2). The total soluble solids, titratable acidity, pH, reducing sugars, total sugars and ascorbic acid were recorded as 2.16 ºB, 0.30 per cent, 4.47, 0.64 per cent, 1.41 per cent and 6.35 mg/100g, respectively. Whereas, Burang (2018) recorded slightly lower value for total soluble solids (1.5 ºB) in *Aloe vera* gel. The findings of Chandegara and Varshney (2013), Jain (2016) and Kiran *et al.* (2017) for the *Aloe vera* gel indicated titratable acidity (0.12 %), total sugars (0.83 %) and 7.89 mg/100g of ascorbic acid, respectively. The gel used in the present study was found to contain 185.53 mg/100g total phenols and 68.21 per cent antioxidant potential. Whereas, Burang (2018) reported 198.70 mg/100g total phenolics and 71.12 per cent antioxidant potential in *Aloe vera* gel. Further, *Aloe vera* gel also contained potassium (0.165 g/100g), phosphorus (0.491 g/100g), manganese (1.60 mg/100g) and magnesium (3.23 mg/100g). Similar range of minerals was also documented by Pandit (2016) in *Aloe vera* gel.



(a) Apple fruits and pulp **(b) *Aloe vera* leaves and gel**

Plate 4.1: Pictorial depiction of (a) fresh apple fruits and apple pulp; (b) mature *Aloe vera* leaves and *Aloe vera* gel

Table 4.2 :Physico-chemical characteristics of fresh apple pulp and *Aloe vera* gel

Parameters*	**Mean ± SE**	
	Apple pulp	***Aloe vera* gel**
A. Physico-chemical and quality characteristics		
Total soluble solids (ºB)	13.62 ± 0.03	2.16 ± 0.10
Titratable acidity (%)	0.49 ± 0.03	0.30 ± 0.01
pH	3.39 ± 0.01	4.47 ± 0.08
Reducing sugars (%)	7.48 ± 0.06	0.64 ± 0.01
Total sugars (%)	12.64 ± 0.14	1.41 ± 0.03
Ascorbic acid (mg/100 g)	10.50 ± 0.26	6.35 ± 0.22
Total phenols (mg/100 g)	40.30 ± 0.10	185.53 ± 0.42
Antioxidant potential (% DPPH free radical scavenging activity)	34.48 ± 0.15	68.21 ± 0.10
B. Minerals		
Potassium (g/100g)	0.162 ± 0.69	0.165 ± 1.22
Phosphorus (g/100g)	0.480 ± 0.91	0.491 ± 0.46
Calcium (mg/100g)	40.00 ± 2.03	11.50 ± 0.52
Copper (mg/100g)	0.43 ± 0.06	0.70 ± 0.02
Iron (mg/100g)	1.22 ± 0.04	0.67 ± 0.13
Manganese (mg/100g)	0.70 ± 0.08	1.60 ± 0.12
Magnesium (mg/100g)	3.21 ± 0.51	3.23 ± 0.20

***** Each value is average of three determinations; SE = Standard Error

4.3 OPTIMIZATION OF PARAMETERS FOR THE DEVELOPMENT OF *ALOE VERA* FORTIFIED APPLE SPREAD USING RESPONSE SURFACE METHODOLOGY (RSM)

4.3.1 Preliminary Experiments for the Preparation of *Aloe vera* Fortified Apple Spread

Keeping in view the negligible literature available on development of *Aloe vera* fortified fruit spread, a preliminary experiment regarding suitability of CMC as gelling agent was conducted. Different combinations of *Aloe vera* pulp and CMC with 0.5 per cent of acid and 60 ºB of TSS were tried as per earlier studies (Holzwarth *et al.,* 2013; Barcelon *et al.,* 2015).

During experimentation, it was observed that carboxymethyl cellulose (CMC) failed to give proper gel like properties to the developed spread (Table 4.3 and Plate 4.2). Therefore, CMC was replaced with food grade pectin for developing the spread. Further, based on the results obtained in the preliminary experiment, different combinations of *Aloe vera* pulp, TSS, acidity and pectin were designed using Response Surface Methodology (RSM) and 30 treatments/runs of spreads were prepared according to the method given in the literature. The responses recorded of each run are given in Table 4.4.

Table 4.3: Preliminary optimization and remarks for the development of *Aloe vera* fortified apple spread

Treatments	*Aloe vera* pulp (%)	CMC (%)	Remarks
T_1	20	0.50	Prepared spread was syrupy and bad in taste
T_2	20	0.75	No gel formation
T_3	20	1.00	Off smell and cloudy gel formation
T_4	30	0.50	Butter like appearance with foul smell
T_5	30	0.75	Released water after five days of production
T_6	30	1.00	Sticky and cloudy product
T_7	40	0.50	Slimy and free flowing product
T_8	40	0.75	Longer cooking time and released free water
T_9	40	1.00	Lump formation and cloudy gel

4.3.2 Physico-chemical and Sensory Responses of *Aloe vera* Fortified Apple Spread

4.3.2.1 Cooking time and Water activity

Data pertaining to effect of different levels of *Aloe vera,* TSS, acidity and pectin on the cooking time and water activity is shown in Table 4.4. It has been observed that the spread produced in the Run 14 took minimum cooking time of 580 seconds which consisted of 10.00 per cent *Aloe vera* pulp, 55.00 ºB TSS, 0.75 per cent acidity, 0.75 per cent pectin. Whereas, spread produced in Run 30 with 50.00 per cent *Aloe vera* pulp, 55.00 ºB TSS, 0.75 per cent acidity and 0.75 per cent pectin, took maximum cooking time of 797 seconds. In case of water activity, the spread developed in Run 11 recorded the lowest water activity (0.7627) while, Run 30 had highest water activity as 0.8290.

The linear terms of *Aloe vera* pulp and TSS influenced cooking time significantly while, pectin was found to be non-significant for both cooking time and water activity (Table 4.5). Even the quadratic terms of *Aloe vera* pulp and TSS and linear term of acidity had significant ($p<0.05$) effect on water activity. Figure 4.1 revealed the effect of *Aloe vera* pulp and TSS on cooking time and water activity which represents that at certain level of TSS, the cooking time of spread increased with increase in concentration of *Aloe vera* pulp. Also, at any level of *Aloe vera* pulp, increase in cooking time was recorded due to increase in TSS.

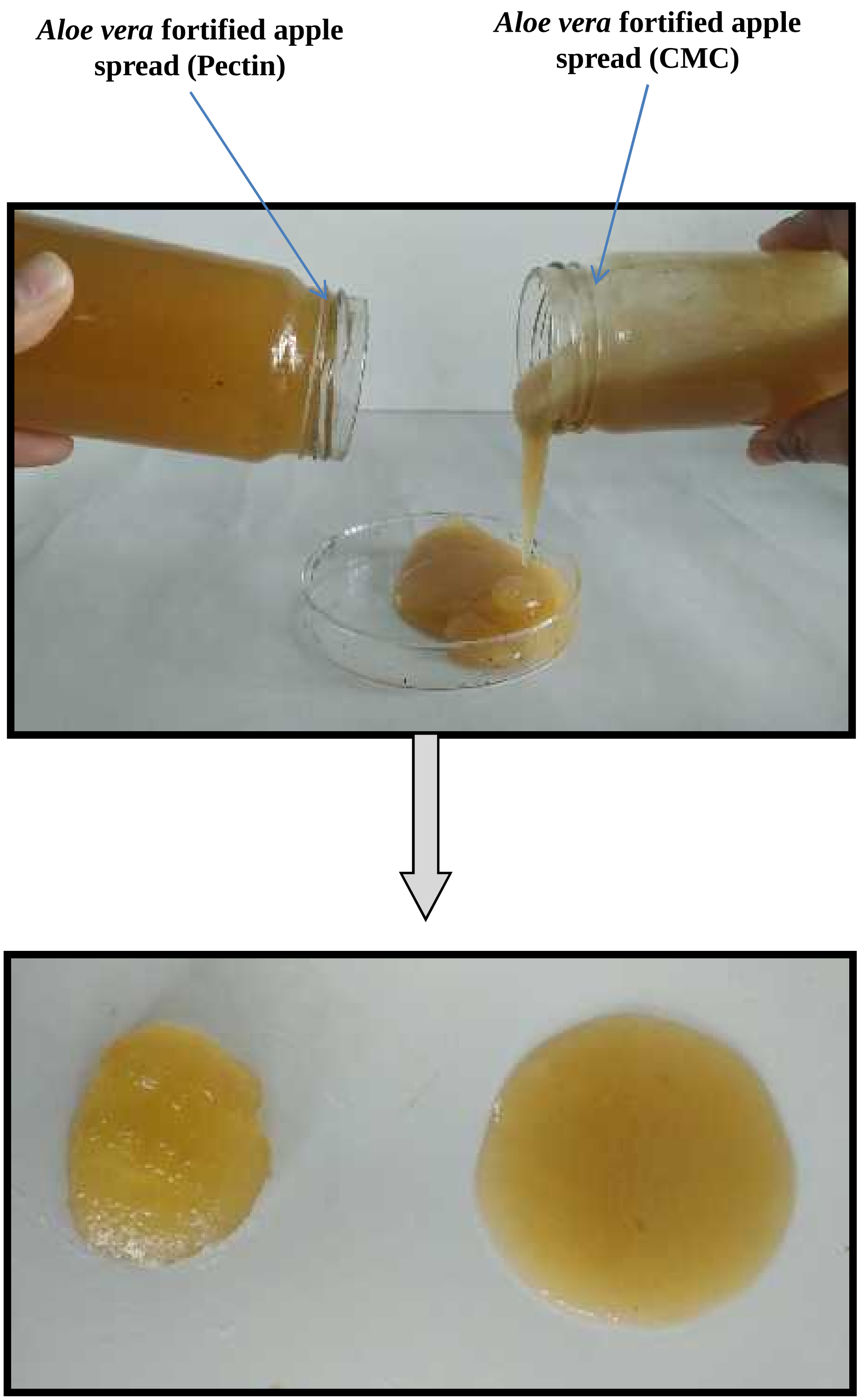


Plate 4.2: Pictorial comparison between *Aloe vera* fortified apple spread prepared with CMC and pectin

Table 4.4: Experimental design and responses obtained for *Aloe vera* fortified apple spread

Run	Variables				Responses							
	Aloe vera pulp (%)	TSS (ºB)	Acidity (%)	Pectin (%)	Cooking time (sec)	Water activity	TSS (ºB)	Spreadability (cm^2/g/min)	Texture	Appearance	Flavour	Overall acceptability
1	30	55	0.75	0.25	670	0.8153	56.0	22.09	7.75	7.50	7.75	7.70
2	20	60	0.50	1.00	648	0.7946	62.0	17.56	7.50	7.25	7.50	7.50
3	20	60	1.00	1.00	640	0.7955	60.5	17.66	7.25	7.00	7.25	7.20
4	30	55	0.25	0.75	655	0.8126	56.0	21.91	7.75	7.50	7.75	7.70
5	30	55	0.75	0.75	660	0.8196	56.0	22.05	7.50	7.25	7.50	7.50
6	40	50	1.00	0.50	668	0.8215	50.5	26.64	8.00	8.00	8.25	8.10
7	30	45	0.75	0.75	645	0.8207	46.0*	25.20	7.50	7.25	7.50	7.50
8	40	50	0.50	0.50	676	0.8209	51.5	26.53	8.25	8.00	8.25	8.20
9	30	55	0.75	0.75	660	0.8196	56.0	22.04	7.50	7.25	7.50	7.50
10	20	50	1.00	0.50	590	0.8199	50.0	20.32	7.25	7.00	7.25	7.20
11	30	65	0.75	0.75	690	0.7627*	66.0**	14.68*	8.00	8.00	8.25	8.10
12	30	55	0.75	0.75	660	0.8196	56.0	22.06	7.50	7.25	7.50	7.50
13	40	50	1.00	1.00	665	0.8284	50.0	26.57	7.75	7.50	7.75	7.70
14	10	55	0.75	0.75	580*	0.8021	55.5	16.15	7.00	6.75	7.00	7.00
15	40	60	0.50	0.50	781	0.8055	60.0	23.34	8.55**	8.75**	8.60**	8.65**
16	30	55	0.75	1.25	625	0.8179	55.0	21.89	6.75*	6.75	6.75*	6.75*
17	30	55	0.75	0.75	660	0.8196	56.0	22.06	7.50	7.25	7.50	7.50
18	30	55	0.75	0.75	660	0.8196	56.0	22.05	7.50	7.25	7.50	7.50
19	20	50	1.00	1.00	615	0.8177	50.0	20.14	7.00	6.50*	7.25	6.90
20	30	55	0.75	0.75	660	0.8196	56.0	22.04	7.50	7.20	7.50	7.40
21	20	60	1.00	0.50	655	0.7959	61.5	17.88	7.50	7.25	7.50	7.50
22	40	60	0.50	1.00	789	0.8059	60.0	23.12	8.25	8.00	8.25	8.20
23	40	60	1.00	0.50	785	0.8070	60.0	23.54	8.50	8.20	8.50	8.40
24	20	50	0.50	1.00	620	0.8107	50.5	19.24	7.00	7.25	7.25	7.10
25	20	50	0.50	0.50	624	0.8146	50.0	19.74	7.25	7.00	7.25	7.20
26	20	60	0.50	0.50	658	0.7975	60.5	18.34	7.75	7.50	7.75	7.70
27	40	50	0.50	1.00	763	0.8276	50.0	26.21	7.50	7.25	7.50	7.50
28	30	55	1.25	0.75	650	0.8271	55.0	22.64	7.00	6.75	7.25	7.00
29	40	60	1.00	1.00	771	0.8057	60.0	23.38	8.00	7.75	8.25	8.00
30	50	55	0.75	0.75	797**	0.8290**	55.0	26.98**	8.00	7.75	8.25	8.00

TSS = Total soluble solids; Sensory score recorded on 9-point Hedonic scale which describes 9 = liked extremely, 1= disliked extremely
*****Minimum, ** maximum

Similar results were also observed by Peinado *et al.* (2013) in strawberry spread, in which the heating time was increased with the increase in TSS and strawberry pulp concentration. Whereas, increase in the concentration of *Aloe vera* pulp (%) has resulted in increased water activity of spreads while, it decreased with increase in TSS level (Figure 4.1). It was also observed that TSS had a great contribution towards water activity which indicated that the high sugar proportion resulted in less availability of free water. In addition, Figure 4.2 also showed that water activity got increased with the per cent increase in acidity and pectin as well. Similar effect was also reported by Peinado *et al.* (2013) in the optimization of strawberry spread. Hua *et al.* (2018) have observed a significant effect on acidity of the polygonatum jam. They observed an increase in water activity with the increase in amount of acid from 0.03 to 0.12 per cent.

The effect of independent variables on cooking time and water activity of *Aloe vera* fortified apple spread was analyzed using analysis of variance (ANOVA) and presented in Table 4.5. The Model F-value of 11.88 and 15.33 of the corresponding responses implied that the model was significant at 5 per cent level. Further, the coefficient of determination (R^2) was observed to be greater than 85 per cent while, lack of fit (LOF) was found to be non-significant. The precision value was greater than 4 which mean that the model can be adequate to describe cooking time and water activity of the spread. The overall second-order polynomial Equation -1 and 3 was adequate to predict the cooking time and water activity of spread and interpret the effect of independent variable obtained by eliminating insignificant terms with p-value more than 0.05 in the Equation - 2 and 4, respectively.

Equation -1: $Y = 660.00 + 53.42 * X_1 + 24.83 * X_2$

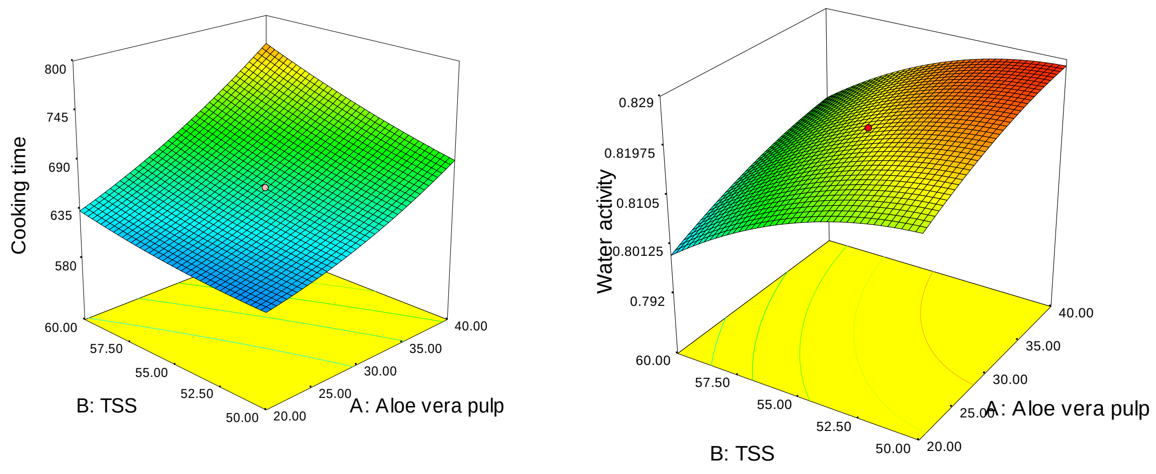
Equation -2 : $Y = 660.00 + 53.42*X1 + 24.83*X2 - 7.50*X3 - 0.67*X4 + 12.63*X1*X2 - 4.37 *X1*X3 + 5.13 *X1*X4 + 7.50 *X2*X3 - 8.50 *X2 *X4 - 5.50 *X3*X4 + 10.50 *X_1^2 + 5.25 *X_2^2 + 1.50 X_3^2 + 0.25 * X_4^2$

Where, Y = Cooking time, X_1 = *Aloe vera* pulp, X_2 = TSS, X_3 = Acidity, X_4 = Pectin

Equation -3: $Y = 0.82 + 5.413*X_1 - 8.737* X_2 + 1.804* X_3 - 1.699 *X_1^2$

Equation -4: $Y = 0.82 + 5.413*X_1 - 8.737*X_2 + 1.804* X_3 + 3.542*X_4 + 3.187*X_1*X_2 - 5.563* X_1*X_3 + 1.381 *X_1*X_4 - 8.188 *X_2*X_3 - 7.313 *X_2*X_4 + 1.687 *X_3*X_4 - 1.699 *X_1^2 - 3.911 *X_2^2 - 6.240 *X_3^2 - 1.436 *X_4^2$

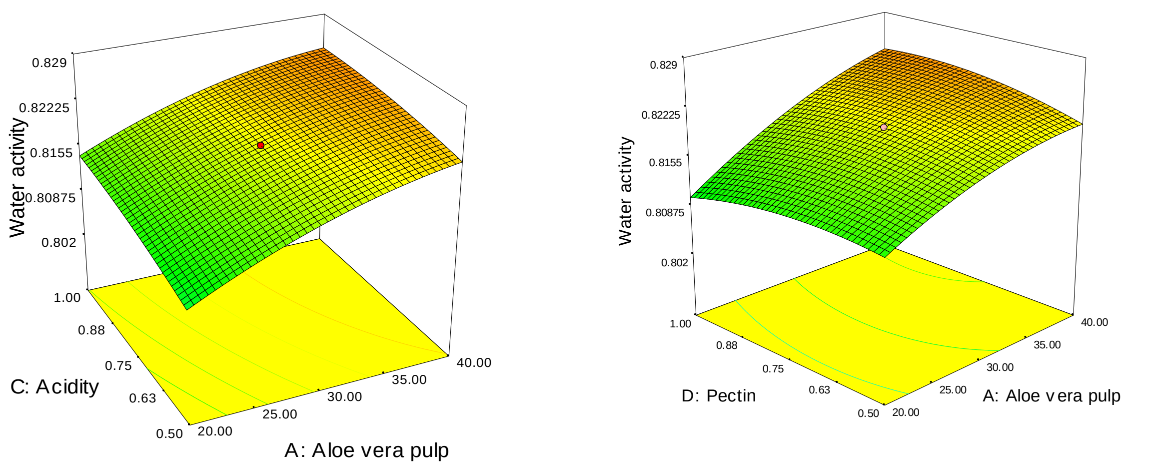
Where, Y = Water activity, X_1 = *Aloe vera* pulp, X_2 = TSS, X_3 = Acidity, X_4 = Pectin



(a)

(b)

Figure 4.1: 3D graph showing the effect of *Aloe vera* pulp and TSS on (a) cooking time; (b) water activity of apple spread



(a)

(b)

Figure 4.2: 3D graph showing the effect of (a) *Aloe vera* pulp and acidity; (b) *Aloe vera* pulp and pectin on the water activity of apple spread

Table 4.5 : Regression coefficient and ANOVA for dependent parameters of *Aloe vera* fortified apple spread

Variables		p-value							
		Cooking Time (sec)	Water activity	TSS (ºB)	Spreadability ($cm^2/g/min$)	Texture	Appearance	Flavour	Overall acceptability
Linear	**X_1**	< 0.0001*	< 0.0001*	0.0590	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*
	X_2	0.0001	< 0.0001	< 0.0001*	< 0.0001*	0.0005*	0.0007*	0.0001*	0.0003*
	X_3	0.1431	0.0356*	0.0590	0.4202	0.0220*	0.0090*	0.1055	0.0155*
	X_4	0.8926	0.6569	0.1463	0.5093	0.0002*	0.0007*	0.0001*	0.0001*
Interactive	**X_1X_2**	0.0507	0.7437	0.0019*	0.2046	0.4068	0.4485	0.1800	0.5451
	X_1X_3	0.4730	0.5698	1.0000	0.9584	0.7799	0.4485	0.4927	0.9031
	X_1X_4	0.4021	0.1696	0.0802	0.7941	0.1754	0.0782	0.0521	0.1283
	X_2X_3	0.2263	0.4058	0.5410	0.5984	0.1754	0.3878	0.1800	0.1933
	X_2X_4	0.1732	0.4567	0.2301	0.9280	0.7799	0.8270	1.0000	0.9031
	X_3X_4	0.3694	0.8624	0.2301	0.7335	0.4068	0.8270	0.4927	0.7155
Quadratic	**X_1^2**	0.0353*	0.0346*	0.0155*	0.7702	0.3080	0.3590	0.0646	0.2742
	X_2^2	0.2655	< 0.0001*	0.7886	0.0169*	0.0224*	0.0069*	0.0016*	0.0076*
	X_3^2	0.7456	0.4068	0.0753	0.4474	0.7605	0.8301	0.2997	0.8734
	X_4^2	0.9568	0.0682	0.0753	0.7330	0.6701	0.8301	0.4548	0.5265
Model F-value		11.88	15.33	280.46	27.39	9.24	8.83	13.72	19.67
R^2		0.92	0.93	0.99	0.96	0.90	0.89	0.93	0.91
Adequate precision		12.71	16.25	16.95	19.15	12.07	11.35	13.53	12.42
LOF		NS	NS	S	NS	NS	NS	NS	NS

TSS= Total soluble solids; Sensory score recorded on 9-point Hedonic scale which describes 9 = liked extremely, 1= disliked extremely

Note : Significant at **p*<0.05; X_1 = *Aloe vera* pulp (%) at linear level, X_2 = TSS (ºB) at linear level, X_3 = Acidity (%) at linear level, X_4 = Pectin (%) at linear level; X_1^2 = *Aloe vera* pulp (%) at quadratic level, X_2^2 =TSS (ºB) at quadratic level, X_3^2 = Acidity (%) at quadratic level, X_4^2 = Pectin (%) at quadratic level; X_1X_2 = Interaction between *Aloe vera* pulp (%) and TSS (ºB), X_1X_3 = Interaction between *Aloe vera* pulp (%) and acidity (%), X_1X_4 = Interaction between *Aloe vera* pulp (%) and pectin (%), X_2X_3 = Interaction between TSS (ºB) and acidity (%), X_2X_4 = Interaction between TSS (ºB) and pectin (%), X_3X_4 = Interaction between acidity (%) and pectin (%); R^2 = Coefficient of determination; LOF = Lack of fit; NS = Non-significant; S= Significant

4.3.2.2 TSS and Spreadability

Table 4.4 reflects the effect of different levels of *Aloe vera* pulp, TSS, acidity and pectin on the TSS and spreadability of developed apple spreads. Minimum value of TSS (46 ºB) and spreadability (14.68 cm^2/g/min) were recorded in the Run 7 and Run 11, whereas, maximum TSS (66 ºB) and spreadability (26.98 cm^2/g/min) were found in the Run 11 and Run 30, respectively. The p-value was used to check the probability of significance of each coefficient. Linear terms of TSS, quadratic terms of *Aloe vera* pulp and interactions of *Aloe vera* pulp and TSS affected the final TSS of the spread significantly ($p<0.05$). Linear level of *Aloe vera* pulp and TSS and quadratic level of TSS had significant effect on spreadability. According to Table 4.5, the effect of acidity and pectin was found to be non-significant in both responses. The TSS of the final product was correlated on the basis of set value in the design. As evident from Figure 4.3, TSS of the spread was increased with the increase in set value of TSS. Whereas, spreadability was directly proportional to the increase in *Aloe vera* pulp due to soft and smooth texture of gel while, it was inversely proportional to increase in TSS as at higher TSS, the spread possess stiffness and jam like consistency (Figure 4.3). Similarly, Jalgaonkar *et al.* (2018) have reported that the increase in sugar syrup and dragon fruit concentration juice, significantly increased the TSS of the dragon fruit beverage. However, Hua *et al.* (2018) have reported that the spreadability was reduced with the increase in viscosity of the jam due to the enhancement of TSS of the jam. Similar findings were also discussed by Garg *et al*. (2018) while, optimizing nutritionally enriched blackberry jam.

Analysis of variance (ANOVA) was used to analyze the significant and non-significant effect of different variables which is presented in Table 4.5. Model was considered to be significant and coefficient of determination (R^2) of TSS and spreadability for the *Aloe vera* fortified apple spread was 99.32 per cent and 96.24 per cent, respectively. Similar regression coefficient (R^2) was also reported by Jalgaonkar *et al.* (2018) for TSS of dragon fruit beverage. The lack of fit was found to be significant for TSS while non-significant for spreadability but precision value was quite high in both the responses i.e. 16.95 and 19.15, respectively. Therefore, the model was considered satisfactory to follow the design space for both the responses of the spread. The overall second-order polynomial equation can be written as Equation - 5 and 7 which was considered adequate to predict the responses and to interpret the effect of *Aloe vera* pulp, TSS, acidity and pectin of spread was obtained by elimination of non-significant terms with p-value more than 0.05 in the Equation - 6 and Equation - 8.

Equation -5 : $Y = 56.00 + 5.08 *X_2 - 0.37 *X_1 *X_2 - 0.21 X_1^2$

Equation -6 : $Y = 56.00 - 0.17 *X_1 + 5.08 *X_2 - 0.17 *X_3 - 0.13 *X_4 - 0.37*X_1*X_2 + 0.000 *X_1 *X_3 - 0.19 *X_1*X_4 + 0.062 *X_2*X_3 + 0.12 *X_3*X_4 - 0.12 *X_1^2 - 0.021 *X_2^2 - 0.15 *X_3^2 - 0.15 *X_4^2$

Where, Y = TSS, X_1= *Aloe vera* pulp, X_2= TSS, X_3= Acidity, X_4 = Pectin

Equation -7 : $Y = 22.05 + 2.92 *X_1 - 1.73 *X_2 - 0.44 *X_2^2$

Equation -8 : $Y = 22.05 + 2.92 *X_1 -1.73 *X_2 + 0.15 *X_3 - 0.12*X_4 - 0.29 *X_1*X_2 - 0.011 *X_1 *X_3 + 0.057 *X_1 *X_4 - 0.12 *X_2*X_3 - 0.020 *X_2 *X_4 + 0.075 *X_3*X_4 - 0.049 *X_1^2 - 0.44 *X_2^2 + 0.33 *X_3^2 + 0.057 *X_4^2$

Where, Y = Spreadability, X_1= *Aloe vera* pulp, X_2= TSS, X_3= Acidity, X_4 = Pectin

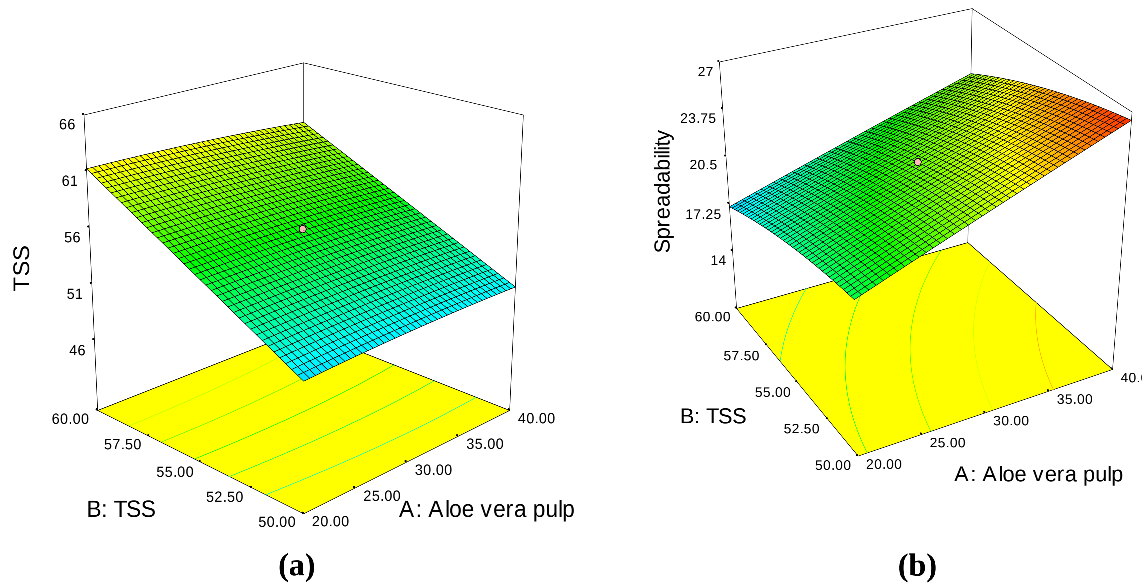


Figure 4.3 : 3D graph showing the effect of *Aloe vera* pulp and TSS on (a) final TSS; (b) spreadability of apple spread

4.3.2.3 Texture and Flavour

The minimum texture score (6.75) and flavour score (6.75) recorded on 9-point Hedonic scale was observed in Run 16 comprised of 30 per cent *Aloe vera* pulp, 55 ºB TSS, 0.75 per cent acidity and 1.25 per cent pectin. Whereas, maximum texture score (8.55) and flavour score (8.60) was found in Run 15 with 40 per cent *Aloe vera* pulp, 60 ºB TSS, 0.5 per cent acidity and 0.5 per cent pectin as shown in Table 4.4. It was observed that the linear level of all the variables and quadratic level of TSS affected the texture.

The linear level effect of *Aloe vera* pulp, TSS and pectin were observed to be highly significant ($p<0.05$) for texture and flavour score, but effect of acidity was found to be non-significant. Figure 4.4 clearly depicts that the texture score increased with the increase in *Aloe vera* pulp and TSS level, while decreased with increase in acidity and pectin of the spread. Similarly, Acosta *et al.* (2008) recorded significant effect of pectin and acidity on the texture of mixed fruit jelly. They found the lowest score ratings for the sample containing high amount of pectin and acid. During gel formation addition of higher amount of acid and lower sugar content may lead to weeping jelly. The improvement in texture of spread might be possible due to increase in TSS of the spread. Addition of *Aloe vera* pulp further helps in maintaining the soft gel of the spread. Further, according to Figure 4.5, the flavour score improved with the increase in both *Aloe vera* pulp from 20 to 40 per cent and TSS from 50 to 60 ºB. On the other hand, flavour scores were decreased with the increase in pectin concentrations (0.5 to 1.0 %).

Table 4.5 reflected the effect of independent variables on texture and flavour of *Aloe vera* fortified apple spread which was evaluated using analysis of variance (ANOVA). The Model F-value of 9.24 and 13.72 of corresponding responses indicated that the model was significant. The R^2 values of texture and flavour for the regression model were 90.06 per cent and 93.11 per cent, respectively. Both the responses had high adequate precision which depicted that the model was well fitted for the spreads. Jayabalan and Karthikeyan (2013) and Faridah *et al.* (2020) found similar range of R^2 value (86.44 to 94.21 per cent) and high precision value which indicated the high level of non-significance of the error. They have reported that the texture and flavour ratings were increased with the increase in sugar level and fruit pulp in the preparation of *Aloe vera* jam and dragon fruit jam, respectively. The overall second-order polynomial equation can be written as Equation - 9 and 11 and considered adequate to predict the both the responses and interpret the effect of *Aloe vera* pulp, TSS, acidity and pectin on it by eliminating the non-significant terms with p-value more than 0.05 in the Equation - 10 and 12.

Equation -9: $Y = 7.50 + 0.36 *X_1 + 0.20 * X_2 – 0.11* X_3 – 0.22* X_4 + 0.11 *X_2^2$

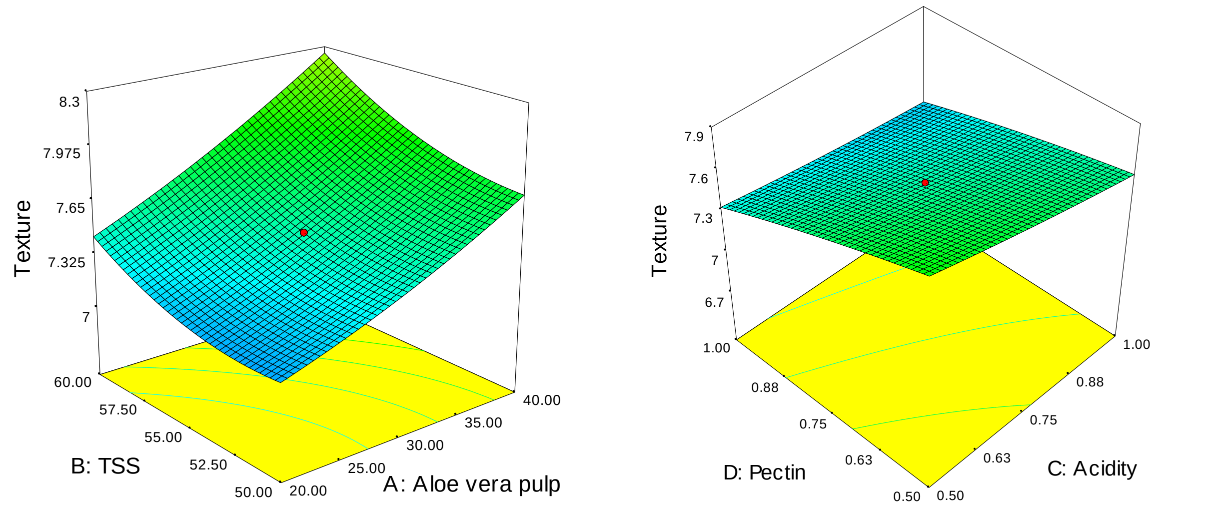
Equation -10: $Y = 7.50 + 0.36 *X_1 + 0.20 * X_2 – 0.11* X_3 – 0.22* X_4 + 0.047 *X_1*X_2 – 0.016 * X_1 *X_3 – 0.078 *X_1*X_4 – 0.078 *X_2*X_3 – 0.016 *X_2*X_4 + 0.047 *X_3*X_4 + 0.044 *X_1^2 + 0.11 *X_2^2 + 0.013 *X_3^2 – 0.018 * X_4^2$

Where, Y = Texture, X_1= *Aloe vera* pulp, X_2= TSS, X_3= Acidity, X_4 = Pectin

Equation -11: $7.50 + 0.38 *X_1 + 0.19 *X_2 – 0.19 *X_4 + 0.13 *X_2^2$

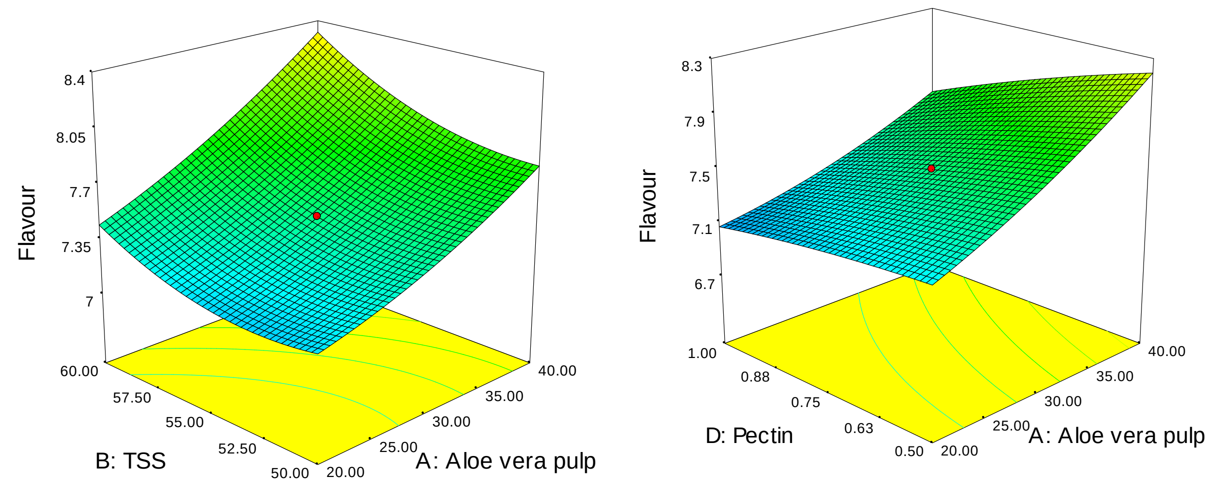
Equation -12: $7.50 + 0.38 *X_1 + 0.19 *X_2 – 0.062 *X_3 – 0.19 *X_4 + 0.062 *X_1*X_2 + 0.031 *X_1*X_3 – 0.094*X_1*X_4 – 0.063 *X_2*X_3 + 0.000*X_2*X_4 + 0.031 *X_3*X_4 + 0.068 *X_1^2 + 0.13 *X_2^2 + 0.036 *X_3^2 – 0.026 *X_4^2$

Where, Y = Flavour, X_1= *Aloe vera* pulp, X_2= TSS, X_3= Acidity, X_4 = Pectin



(a) **(b)**

Figure 4.4 : 3D graph showing the effect of (a) *Aloe vera* pulp and TSS; (b)acidity and pectin on texture of apple spread



(a) **(b)**

Figure 4.5: 3D graph showing the effect of (a) *Aloe vera* pulp and TSS; (b) *Aloe vera* pulp and pectin on flavour score of apple spread

4.3.2.4 Appearance and Overall acceptability

The experimental results with respect to effect of variations in the level of *Aloe vera* pulp, TSS, acidity and pectin on the appearance and overall acceptability score of spread are presented in Table 4.4. It was observed that the spread prepared in the Run 19 and Run 16 resulted in the minimum score for appearance and overall acceptability as 6.50 and 6.75, respectively. However, maximum scores of 8.75 and 8.65 for appearance and overall acceptability, respectively were noticed in Run 15 containing 40 per cent *Aloe vera* pulp, 60 ºB TSS, 0.50 per cent acidity and 0.50 per cent pectin.

Analysis of data also indicated that linear term of *Aloe vera* pulp, TSS, acidity and pectin and quadratic term of TSS were observed to influence the appearance and overall acceptability score significantly ($p<0.05$). As shown in Figure 4.6, the appearance score increased with the increase in levels of *Aloe vera* pulp and TSS while, decreased with the increase in the acidity and pectin content in *Aloe vera* fortified apple spread. During preparation of spread, it was observed that addition of higher amount of sugar and *Aloe vera* pulp provided bright colour to the spread, while higher acidity and pectin resulted in cloudiness of the product. Similarly, according to Singh and Bunkar (2015), the appearance score of pomegranate-ginger drink increased with the increase in sugar and fruit juice content. Further, the effect of *Aloe vera* pulp and TSS on overall acceptability (Figure 4.7) revealed that it increased with increase in level of *Aloe vera* pulp and TSS from 20 to 40 per cent and 50 to 60 ºB, respectively. Whereas, a decreasing trend in overall acceptability score with increase in the concentration of acidity and pectin was observed (Figure 4.7). Parsayee *et al.* (2013) have also recorded similar decrease in overall acceptability with increase in pectin in low calorie sour cherry jam.

The effect of *Aloe vera* pulp, TSS, acidity and pectin on appearance of *Aloe vera* fortified apple spread was analyzed using analysis of variance (ANOVA) and presented in Table 4.5. Coefficient of determination (R^2) for the regression model of appearance was 89.18 per cent and for overall acceptability was 91.48 per cent. In both the responses, the lack of fit was found to be non-significant and the precision value was quite high i.e. more than 4. Therefore, the model was considered satisfactory to follow the design space for appearance and overall acceptability of the spread. Singh and Bunkar (2015) reported approximately similar R^2 value (87.01) with adequate precision of 14.64 in pomegranate-ginger drink for appearance score which means that the model was desirably fit to the design. The overall second-order polynomial equation can be written as Equation - 13 and 14, and considered adequate to calculate both responses of the spread and understand the effect of all the variables that was obtained by elimination of non- significant terms with p-value more than 0.05 in Equation - 15 and 16.

Equation -13: $7.24 + 0.36 *X_1 + 0.20 *X_2 – 0.14 *X_3 – 0.20 *X_4 + 0.13 *X_2^2$

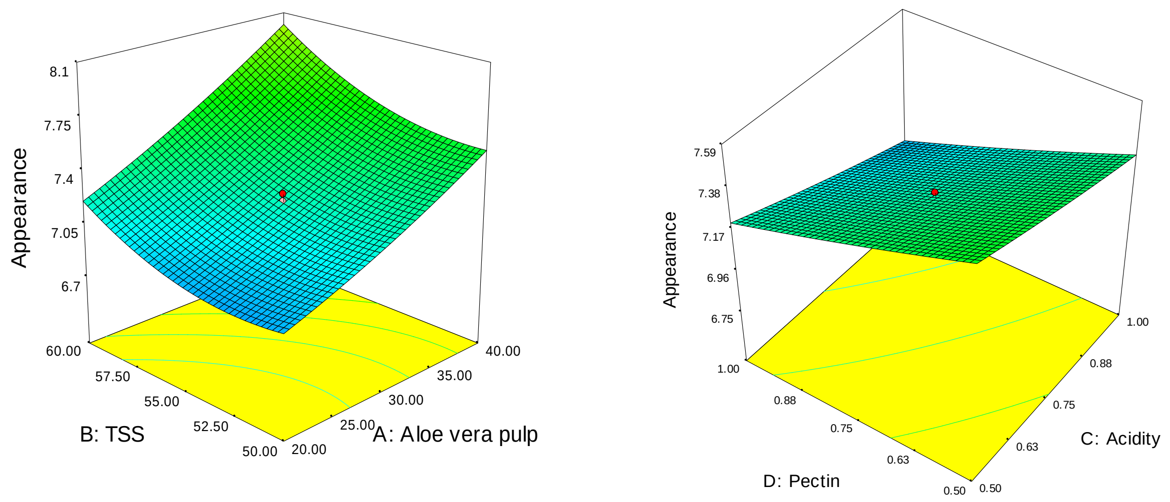
Equation -14: $7.24 + 0.36 *X_1 + 0.20 *X_2 – 0.14 *X_3 – 0.20 *X_4 + 0.044 *X_1*X_2 + 0.044 *X_1*X_3 – 0.11 *X_1*X_4 – 0.050 *X_2*X_3 – 0.013 *X_2*X_4 – 0.013 *X_3*X_4 + 0.041 *X_1^2 + 0.13 X_2^2 + 9.375 *X_3^2 + 9.375 *X_4^2$

Where, Y = Appearance, X_1= *Aloe vera* pulp, X_2= TSS, X_3= Acidity, X_4 =Pectin

Equation -15: $7.48 + 0.36 *X_1 + 0.20 *X_2 – 0.11 * X_3 – 0.21 *X_4 + 0.12 *X_2^2$

Equation -16: $7.48 + 0.36 *X_1 + 0.20 *X_2 – 0.11 * X_3 – 0.21 *X_4 + 0.031 *X_1*X_2 + 6.250 *X_1*X_3 – 0.081 *X_1*X_4 – 0.069 *X_2*X_3 – 6.250 *X_2*X_4 + 0.019 *X_3*X_4 + 0.044 *X_1^2 + 0.12 *X_2^2 + 6.250 * X_3^2 – 0.025 * X_4^2$

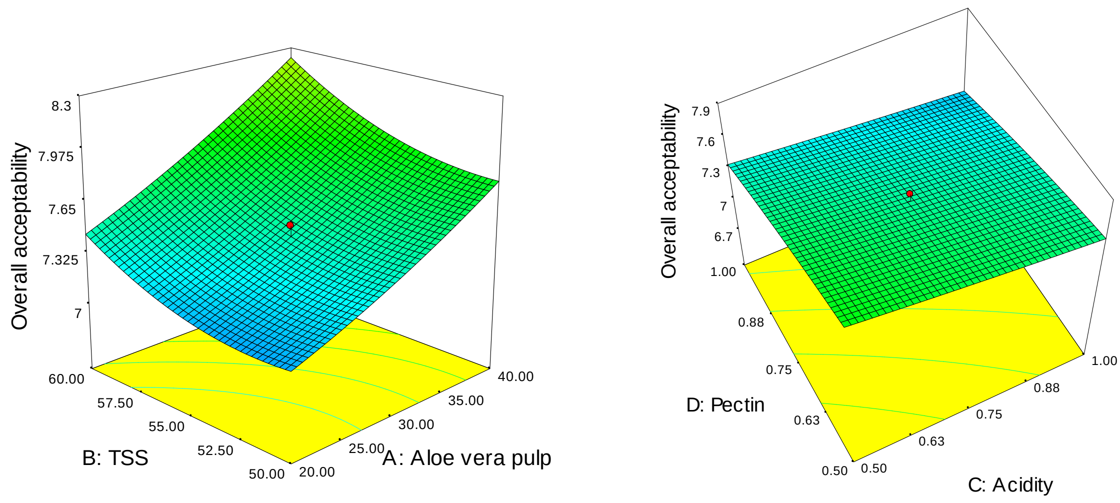
Where, Y = Overall acceptability, X_1= *Aloe vera* pulp, X_2= TSS, X_3= Acidity, X_4 = Pectin



(a) **(b)**

Figure 4.6: 3D graph showing the effect of (a) *Aloe vera* pulp and TSS; (b) acidity and pectin on appearance score of apple spread

Goals were set for each independent variable and responses before optimization as given in Table 4.6. The independent variables i.e. acidity and pectin were kept in range while maximum level of *Aloe vera* pulp and overall acceptability was set. TSS was set up to the target 60 °B which were based on the results obtained during preliminary experiments so that an optimum product could be obtained with respect to its desirable composition and functional properties. Responses such as cooking time, spreadability, texture, appearance and flavour were set in range because the desired response was achieved at minimum value. But in case of water activity and overall acceptability goals were set in range and maximum, respectively.



(a) **(b)**

Figure 4.7: 3D graph showing the effect of (a) *Aloe vera* pulp and TSS; (b) pectin and acidity on overall acceptability of apple spread

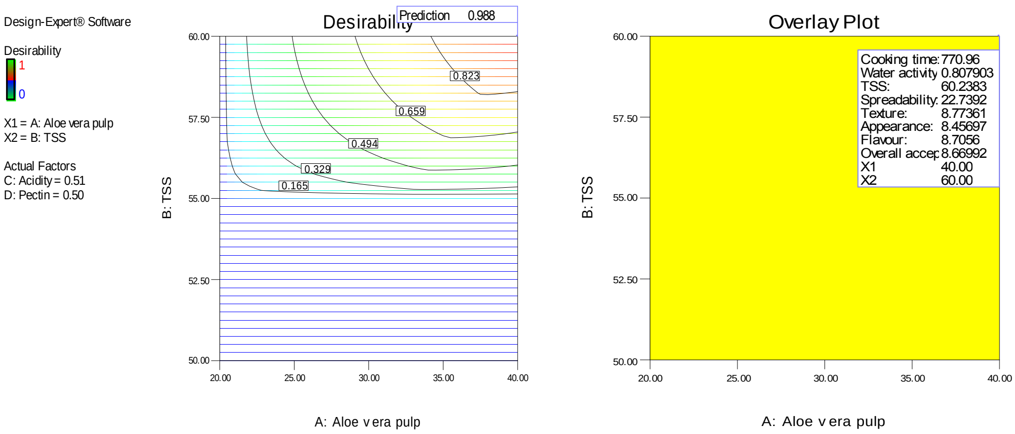
Table 4.6: Goals for optimization of formulations to prepare *Aloe vera* fortified apple spread

Name	Goal	Lower Limit	Upper Limit
Aloe vera pulp (%)	Maximum	20.00	40.00
TSS (ºB)	target = 60.00	55.00	60.00
Acidity (%)	in range	0.50	1.00
Pectin (%)	in range	0.50	1.00
Cooking time (sec)	in range	580	797
Water activity	minimum	0.76	0.83
Spreadability (cm^2/g/min)	in range	14.68	26.98
Texture	in range	6.75	9.00
Appearance	in range	6.50	8.75
Flavour	in range	6.75	8.75
Overall acceptability	maximum	6.75	8.65

Based on the above goals, the RSM suggested solutions for optimization of process (Appendix-V). Perusal of data in Figure 4.8 indicated that the best selected conditions were i.e. *Aloe vera* pulp, TSS, acidity and pectin 40 per cent, 60 ºB, 0.51 per cent and 0.50 per cent, respectively which had the highest desirability of the fit i.e. 0.988, which was found to be quite good. A confirmatory test was conducted for the optimized levels of ingredients for preparation of *Aloe vera* fortified apple spread and the responses studied. The predicted values of variables and the result recorded for responses were compared with the observed value using t-test. The significance of difference between predicted values and observed values during preparation of *Aloe vera* fortified apple spread is given in Table 4.7. No significant difference was observed between predicted values and observed values of the responses, therefore, these conditions were found to be suitable for the preparation of *Aloe vera* fortified apple spread and used for the development of *Aloe vera* fortified low calorie apple spread.

Table 4.7: Predicted and observed values of responses of *Aloe vera* fortified apple spread

Sr. No.	Responses	Predicted value	Observed value	t-test
1.	Cooking time (sec)	771	775	NS
2.	Water activity	0.81	0.78	NS
3.	TSS (ºB)	60.20	60.60	NS
4.	Spreadability ($cm^2/g/min$)	22.78	22.57	NS
5.	Texture	8.81	8.74	NS
6.	Appearance	8.48	8.50	NS
7.	Flavour	8.73	8.75	NS
8.	Overall acceptability	8.70	8.73	NS



(a) **(b)**

Figure 4.8: (a) contour graph; (b) overlay plot showing the effect of interaction of *Aloe vera* pulp and TSS on the desirability of apple spread

4.4 OPTIMIZATION OF CONCENTRATIONS OF SWEETOS (FOS-SUCRALOSE) AND PECTIN FOR THE DEVELOPMENT OF *ALOE VERA* FORTIFIED LOW CALORIE APPLE SPREAD

The suitability of developing *Aloe vera* fortified low-calorie functional apple spread was evaluated by replacing sugar (sucrose) with sweetos (% equivalent sweetness with sucrose). Data pertaining to the effect of different concentrations of sweetos (FOS-sucralose) on various sensory characteristics of the prepared spread is presented in Table 4.8 and discussed as under:

The addition of sweetos for the replacement of sucrose in *Aloe vera* fortified apple spread brought significant changes on sensory characteristics of the final spread. The mean score for appearance among different products ranged between 7.0 and 8.00 (Table 4.8) on 9-point Hedonic scale. However, appearance score was observed to increase significantly with increase in concentration of sweetos from 25 to 100 per cent. It was highest (8.00) in 100 per cent sweetos with 0.5 per cent pectin (T_{11}), while, lowest score (7.0) was recorded for 100 per cent sucrose with 0.5 per cent pectin level. The samples with high sugar levels were found to be darker in appearance due to maillard reaction. Whereas, the samples prepared with high sweetos level had light and glossy appearance which was '*liked very much'* by the judges (Plate 4.3). Similar observations were also recorded by Muhammad *et al.* (2008) in low calorie apple fruit jam and Pielak *et al.* (2020) in low sugar apple preserve.

The texture score of sweetos sweetened *Aloe vera* fortified low calorie apple spread was observed to decrease with increase in proportion of pectin and sweetos (Plate 4.3). The maximum texture score (8.70) was observed in the spread (T_{11}) prepared with 100 per cent of sweetos and 0.5 per cent pectin, while, minimum score (6.7) was noticed in the spread containing 75 per cent sucrose, 25 per cent sweetos and 1.5 per cent pectin i.e. T_4. It was found that the spreads containing low concentration of pectin had smooth and desirable texture as compared to the spreads with high concentration of pectin.

Data pertaining to the effect of different concentrations of sweetos and pectin on spreadability rating of *Aloe vera* fortified low-calorie apple spread are presented in Table 4.8. The spreadability score was recorded to range from 6.55 to 8.10. The spreadability score recorded on 9-point Hedonic scale decreased with the increase in pectin content beyond 1.0 per cent level which produced stiff gel that affected uniform spreadability of the product on the bread. The evaluation score of the product was in conformity with earlier studies conducted by Barcelon *et al.* (2015) in white and red dragon fruit spread. Furthermore, the treatment T_{11} having highest spreadability score (8.10) was found to be statistically at par with treatments T_5, T_8 and T_{12}.

Flavour score of prepared spreads exhibited a gradual increase with substitution of sucrose with sweetos and increase in pectin concentration. The treatment T_{13} (100 % Sw) with 1.5 per cent pectin obtained highest score (8.33) and T_2 (75 % Su + 25 % Sw) with 0.5 per cent pectin got lowest score (8.00). While, the treatments T_{11} and T_{12} were found to be statistically ($p<0.05$) at par with treatment T_{13}. These findings were also in conformity with the inferences of Sutwal *et al.* (2019) in the preparation of stevia sweetened low calorie apple jam.

Table 4.8 Effect of different concentrations of sweetos and pectin on sensory characteristics of *Aloe* vera fortified low calorie apple spreads

Treatments	**Sensory score ***				
	Appearance	**Texture**	**Spreadability**	**Flavour**	**Overall acceptability**
T_1 (100 % sucrose)	7.00	7.50	7.30	8.05	7.25
T_2 (75 % Su + 25 % Sw + 0.5% Pe)	7.09	7.53	7.30	8.00	7.20
T_3 (75 % Su + 25 % Sw + 1.0% Pe)	7.20	7.10	7.00	8.02	7.00
T_4 (75 % Su + 25 % Sw + 1.5% Pe)	7.21	6.70	6.55	8.05	6.80
T_5 (50 % Su + 50 % Sw + 0.5% Pe)	7.24	7.76	7.50	8.08	7.50
T_6 (50 % Su + 50 % Sw + 1.0% Pe)	7.28	7.50	7.10	8.09	7.40
T_7 (50 % Su + 50 % Sw+ 1.5% Pe)	7.30	7.30	7.12	8.12	7.20
T_8 (25 % Su + 75 % Sw + 0.5% Pe)	7.35	8.20	8.05	8.20	7.80
T_9 (25 % Su + 75 % Sw + 1.0% Pe)	7.39	8.00	7.35	8.23	7.10
T_{10} (25 % Su + 75 % Sw + 1.5% Pe)	7.14	6.75	6.60	8.08	6.90
T_{11} (100 % Sw + 0.5% Pe)	8.00	8.70	8.10	8.30	8.00
T_{12} (100 % Sw + 1.0% Pe)	7.54	8.40	7.85	8.31	7.71
T_{13} (100 % Sw + 0.5% Pe)	7.55	8.00	7.35	8.33	7.69
$CD_{0.05}$	0.06	0.47	0.61	0.05	0.20

*Sensory score recorded on 9-point Hedonic scale which describes 9 = liked extremely, 1= disliked extremely; Su = Sucrose, Sw = Sweetos, Pe = Pectin

Data in Table 4.8 revealed that the overall acceptability score of spreads decreased with increase in pectin and sweetos level. The treatment T_{11} was noticed superior with the score 8.00 while, lowest score (6.80) was found in the treatment T_4. The addition of sweetos with 0.5 per cent of pectin provided better spread as compared to the spread sweetened with the sucrose (control). Similar scores were recorded by Muhammad *et al.* (2008) in the diet apple jam. It was concluded that the *Aloe vera* fortified low calorie apple spread with 100 per cent sweetos and 0.5 per cent pectin (T_{11}) was significantly superior, hence was optimized and subjected to the physico-chemical evaluation.

4.5 PHYSICO-CHEMICAL AND SENSORY CHARACTERISTICS OF APPLE SPREAD, *ALOE VERA* FORTIFIED APPLE SPREAD AND LOW CALORIE APPLE SPREAD

Best rated *Aloe vera* fortified apple spread optimized by RSM in Experiment 4.3 and best rated low calorie apple spread standardized by sensory evaluation in Experiment 4.4 were selected for comparison of physico-chemical and nutritional data described in Table 4.9 and Figure 4.9 indicated that the fortification of *Aloe vera* has improved the therapeutic and nutritional quality of spread. The water activity of the spreads fell under desirable limits i.e. 0.75 in apple spread, 0.78 in *Aloe vera* fortified apple spread and 0.81 in low calorie spread. Similar water activity range was also reported by Abid *et al.* (2017) in pomegranate jam. The difference in the water activity might be due to the moisture difference between spreads. The non-enzymatic browning in *Aloe vera* fortified apple spread is comparatively lesser might be due to the addition of *Aloe vera* pulp (40%). Apple spread and *Aloe vera* fortified apple spread contained higher percentage of total sugars but lesser reducing sugars in comparison to that of low calorie apple spread. The possible reason for this might be that sweetos (fructooligosaccharide-sucralose) contained fructose (reducing sugars) units in their chemical structures with very less quantity of non-reducing sugars. Further, total energy value in low calorie spread (100.27 Kcal/100g) was very less as compared to *Aloe vera* fortified apple spread and apple spread which containing 100 per cent sucrose (Figure 4.9). It might be due to the fact that sweeteners like fructooligosaccharide provide about 1.0 to 1.7 Kcal/g energy in the food products (Khanvilkar and Arya, 2015) and suralose is zero calorie sweetener (Ansari *et al.*, 2015). Similar value was also revealed in the plum squash sweetened with sorbitol (Barwal *et al.*, 2002) and orange based low calorie jam sweetened with stevioside and sucralose (Abolila *et al.*, 2015). The total phenols of *Aloe vera* fortified apple spread (77.27 mg/100g) and low calorie apple spread (74.66 mg/100g) were higher as compared to apple spread (36.69 mg/100g).

Table 4.9 Physico-chemical, minerals and sensory attributes of apple spread and *Aloe vera* fortified apple spreads

Parameters*	**Mean ±SE**		
	Apple spread (100% Ap, 100 % Su)	***Aloe vera* fortified apple spread (40% Al, 100% Su)**	***Aloe vera* fortified low calorie apple spread (40% Al, 100% Sw)**
A. Physico-chemical and nutritional characteristics			
Visual colour	Greyed orange 165 B group	Greyed orange 164 A group	Greyed orange 164 B group
Water activity	0.75 ± 0.04	0.78 ± 0.02	0.81 ± 0.02
Moisture (%)	24.04 ± 0.86	27.39 ± 0.05	29.76 ± 0.93
TSS (ºB)	60.8 ± 1.19	60.60 ± 2.02	51.48 ± 1.94
Titratable acidity (%)	0.54 ± 0.03	0.57 ± 0.07	0.60 ± 0.04
pH	3.65 ± 0.53	3.46 ± 0.10	3.00 ± 0.05
Reducing sugars (%)	22.92 ± 0.83	24.19 ± 0.92	29.10 ± 1.22
Total sugars (%)	55.83 ± 2.19	58.66 ± 1.36	42.07 ± 1.65
Non-enzymatic browning (NEB)	0.45 ± 0.03	0.34 ± 0.07	0.26 ± 0.02
Fat (%)	2.28 ± 0.06	1.71 ± 0.49	1.25 ± 0.06
Protein (%)	2.16 ± 0.75	1.84 ± 0.35	1.08 ± 0.09
Ash content (%)	1.93 ± 0.61	1.03 ± 0.17	1.52 ± 0.36
Ascorbic acid content (mg/100g)	14.23 ± 1.35	12.39 ± 0.96	13.05 ± 0.70
Total phenols (mg/100g)	36.69 ± 3.83	77.27 ± 1.92	74.66 ± 3.24
Antioxidant potential (% DPPH free radical scavenging activity)	31.51 ± 0.11	55.46 ± 1.39	53.67 ± 2.22
Energy value (Kcal/100g)	306.6 ± 0.79	291.63 ± 1.20	100.27 ± 1.43
B. Minerals			
Potassium (g/100g)	0.158 ± 1.73	0.163 ± 0.12	0.162 ± 3.23
Phosphorus (g/100g)	0.478 ± 0.42	0.489 ± 0.08	0.487 ± 1.04
Calcium (mg/100g)	38.50 ± 2.48	09.96 ± 0.52	10.20 ± 1.64
Copper (mg/100g)	0.39 ± 0.06	0.67 ± 0.02	0.66 ± 0.06
Iron (mg/100g)	1.08 ± 0.91	0.55 ± 0.07	0.58 ± 0.04
Manganese (mg/100g)	0.60 ± 0.17	1.56 ± 0.06	1.54 ± 0.01
Magnesium (mg/100g)	2.96 ± 0.62	3.05 ± 0.24	3.03 ± 0.60
C. Sensory Characteristics			
Appearance	7.50 ± 0.26	8.50 ± 0.33	8.00 ± 0.30
Texture	7.00 ± 0.15	8.74 ± 0.45	8.70 ± 0.32
Spreadability	6.50 ± 0.21	8.25 ± 0.10	8.10 ± 0.25
Flavour	8.00 ± 0.08	8.75 ± 0.32	8.30 ± 0.09
Overall acceptability	7.50 ± 0.24	8.73 ± 0.07	8.00 ± 0.14

*Each parameters is the average of three determinations; SE = Standard Error, Ap = Apple, Al = *Aloe vera*, Su = Sucrose, Sw = Sweetos

Plate 4.3: Low calorie *Aloe vera* fortified apple spreads with varying concentrations (0, 25, 50, 75, 100 % sweetness level) of sweetos

Plate 4.4 : Apple spread and *Aloe vera* fortified apple spreads

Similarly, comparatively higher antioxidants activity of *Aloe vera* fortified apple spread (55.46 per cent) and low calorie apple spread (53.67 per cent) were observed while, very less amount was obtained in apple spread as 31.51 per cent (Figure 4.9).

The above results are in conformity with the inferences recorded by Alsuhaibani and Al-Kuraieef (2018) and Sutwal *et al*. (2019) in low calorie pumpkin jam and apple jam, respectively. However, the potassium and phosphorus in *Aloe vera* fortified apple spread (0.163 and 0.489 g/100g) and low calorie apple spread (0.162 and 0.487 mg/100g) were higher as compared to apple spread (0.158 and 0.478 g/100g), respectively. Also, copper, manganese and magnesium content in *Aloe vera* fortified spreads was higher as compared to control sample as given in the Figure 4.10.

Further, the aloin content in different *Aloe vera* fortified apple spreads was detected by using qualitative method of determination i.e. thin layer chromatography (TLC). As shown in the Plate 4.5 (a-b), in TLC estimation did not get any spot in the samples corresponding to the spot of aloin standard (R_1) which revealed that aloin was absent in the samples (spreads). It was observed that combination of ethyl acetate, methanol and water gave maximum separation of *Aloe vera* constituents. The proportion of *Aloe vera* was less in the products, which had reduced the chances of high aloin content in the samples, T_1 (apple spread), T_2 (*Aloe vera* fortified apple spread) and T_3 (low calorie apple spread). The acceptable limit for aloin content in the should be $\leq$ 10 ppm as prescribed for orally ingested *Aloe vera* based products (Pandey *et al.*, 2016).

Further, addition of *Aloe vera* also exerted acceptable effects on sensory attributes of the product developed. The *Aloe vera* fortified apple spreads had recorded higher score for appearance (8.50), texture (8.74), spreadability (8.25) and flavour (8.75) as compared to apple spread and low calorie spread (Figure 4.11). However, the sensory scores recorded for low calorie spread were at par with that of *Aloe vera* fortified apple spread which means that it was equally accepted by panelists (Table 4.9). Data pertaining to spreadability of the product clearly depicts that incorporation of *Aloe vera* has increased the spreadability which is the most important parameter considered while optimizing a best combination. A comparative overview on spreadability of different spreads on the bread pieces is depicted in Plate 4.6 (a-b-c). Similar sensory scores were revealed by many scientists in fruit spreads (Holzwarth *et al.*, 2013; Barcelon *et al.*, 2015; Sorour *et al.*, 2016).

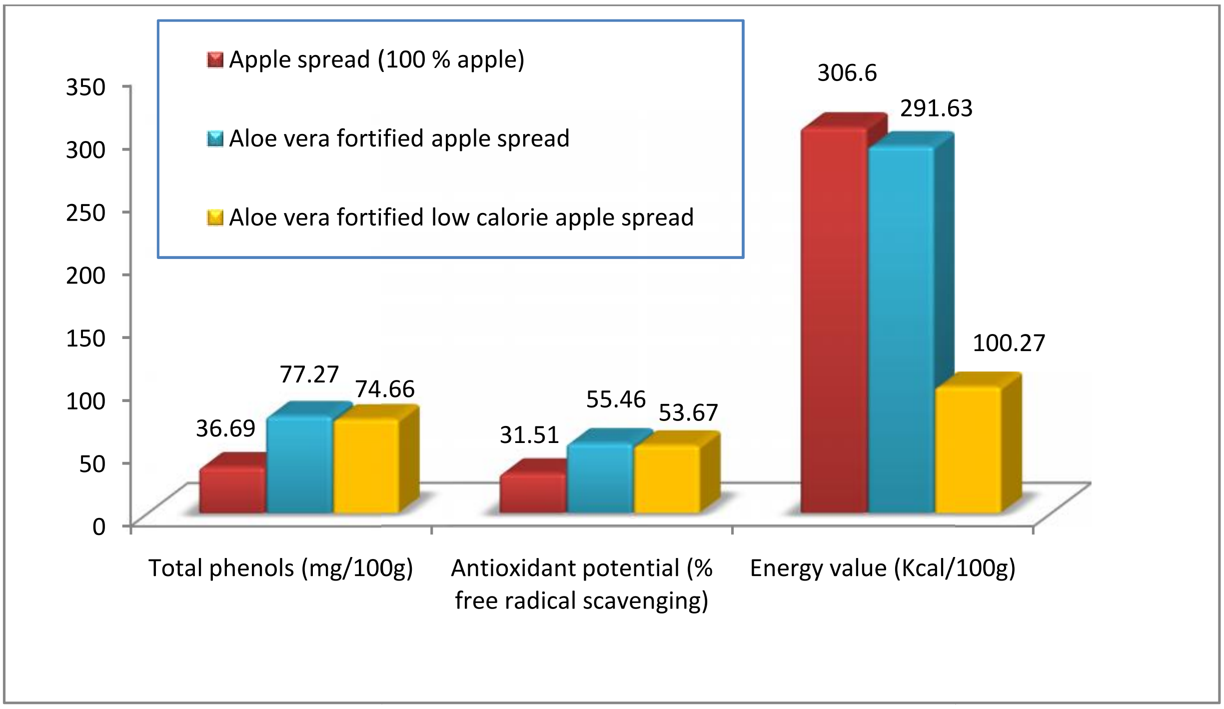


Figure 4.9 : Comparison of nutritive and antioxidant activity of apple spread and *Aloe vera* fortified apple spreads

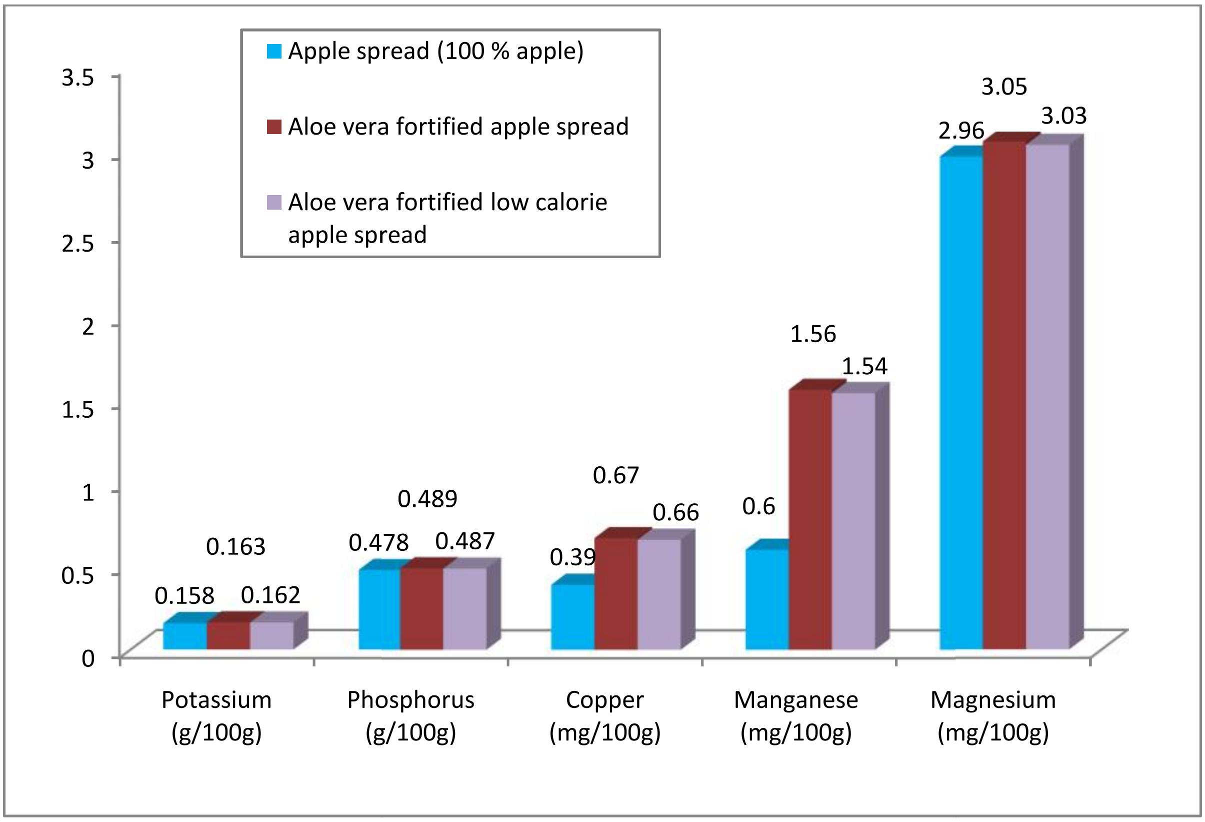
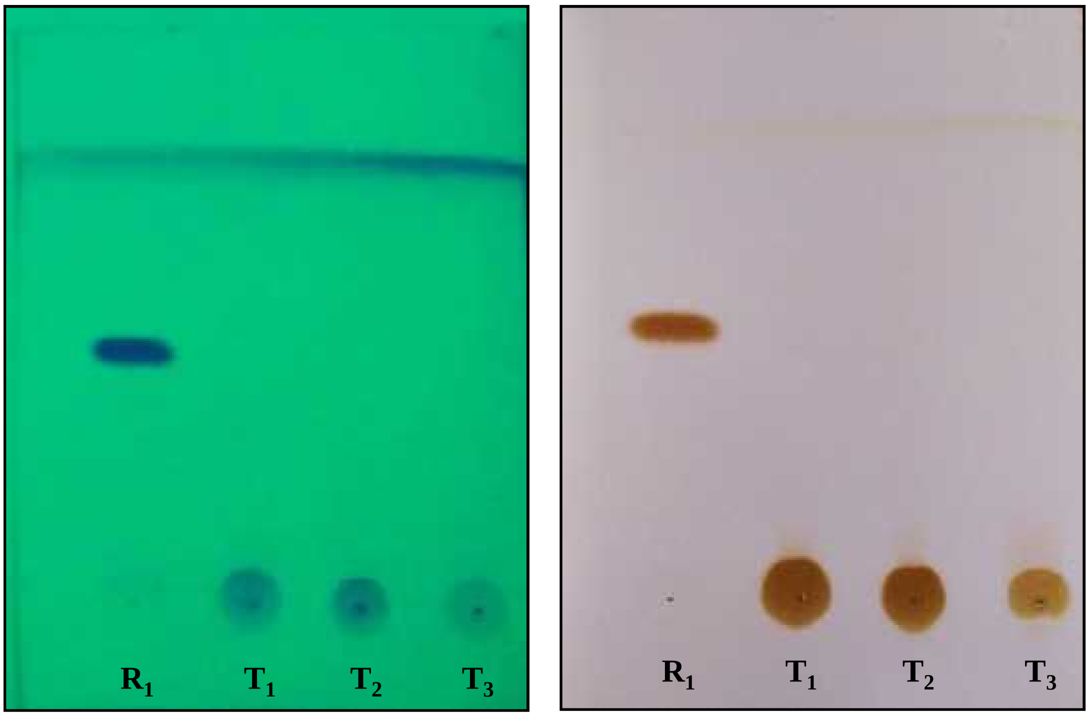


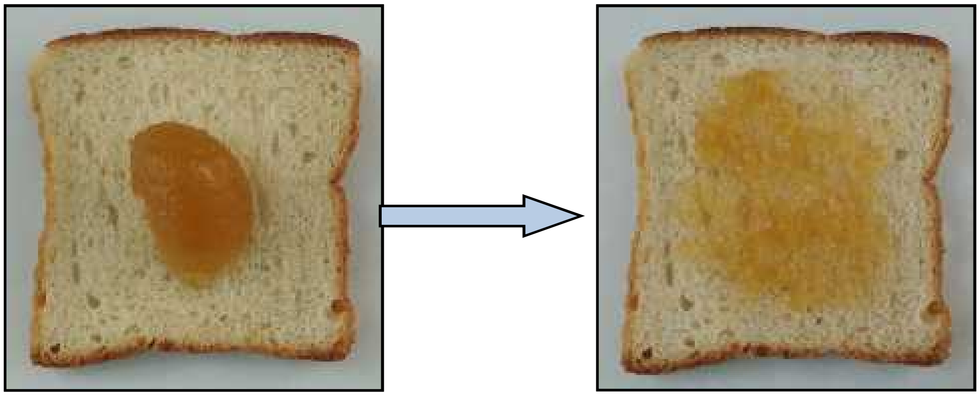
Figure 4.10: Comparison of minerals content in apple spread and *Aloe vera* fortified apple spreads



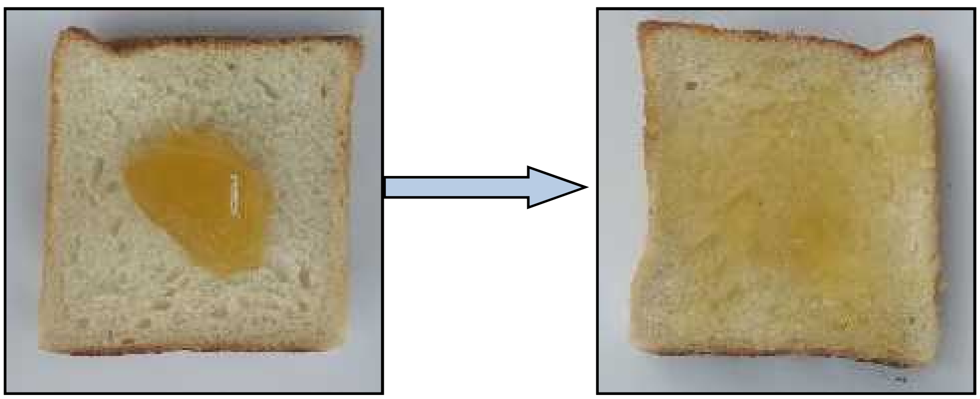
(a) **(b)**

Where, R_1 = Reference compound (aloin)
T_1= Apple spread (control)
T_2 = *Aloe vera* fortified apple spread
T_3 = Low calorie apple spread

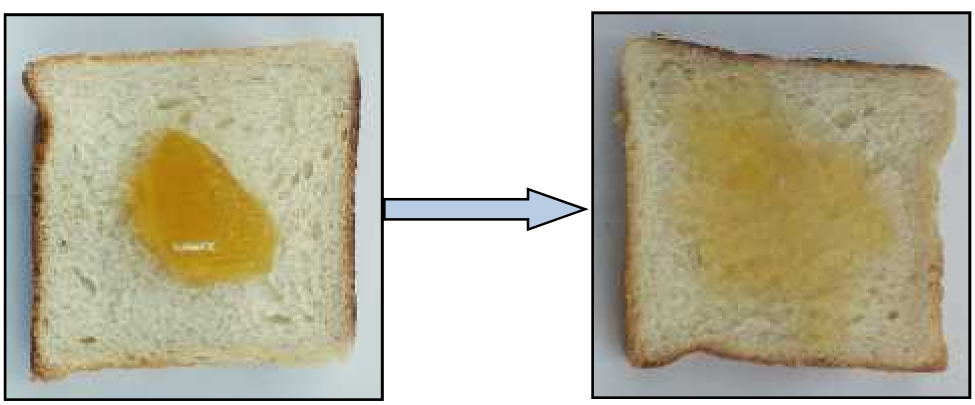
Plate 4.5 (a-b): UV light and alcohol sprayed TLC illustration of aloin content in *Aloe vera* fortified apple spreads



a) Apple spread



b) *Aloe vera* fortified apple spread



c) *Aloe vera* fortified low calorie apple spread

Plate 4.6 (a-b-c): Spreadability of apple spread; *Aloe vera* fortified apple spread and *Aloe vera* fortified low calorie apple spread

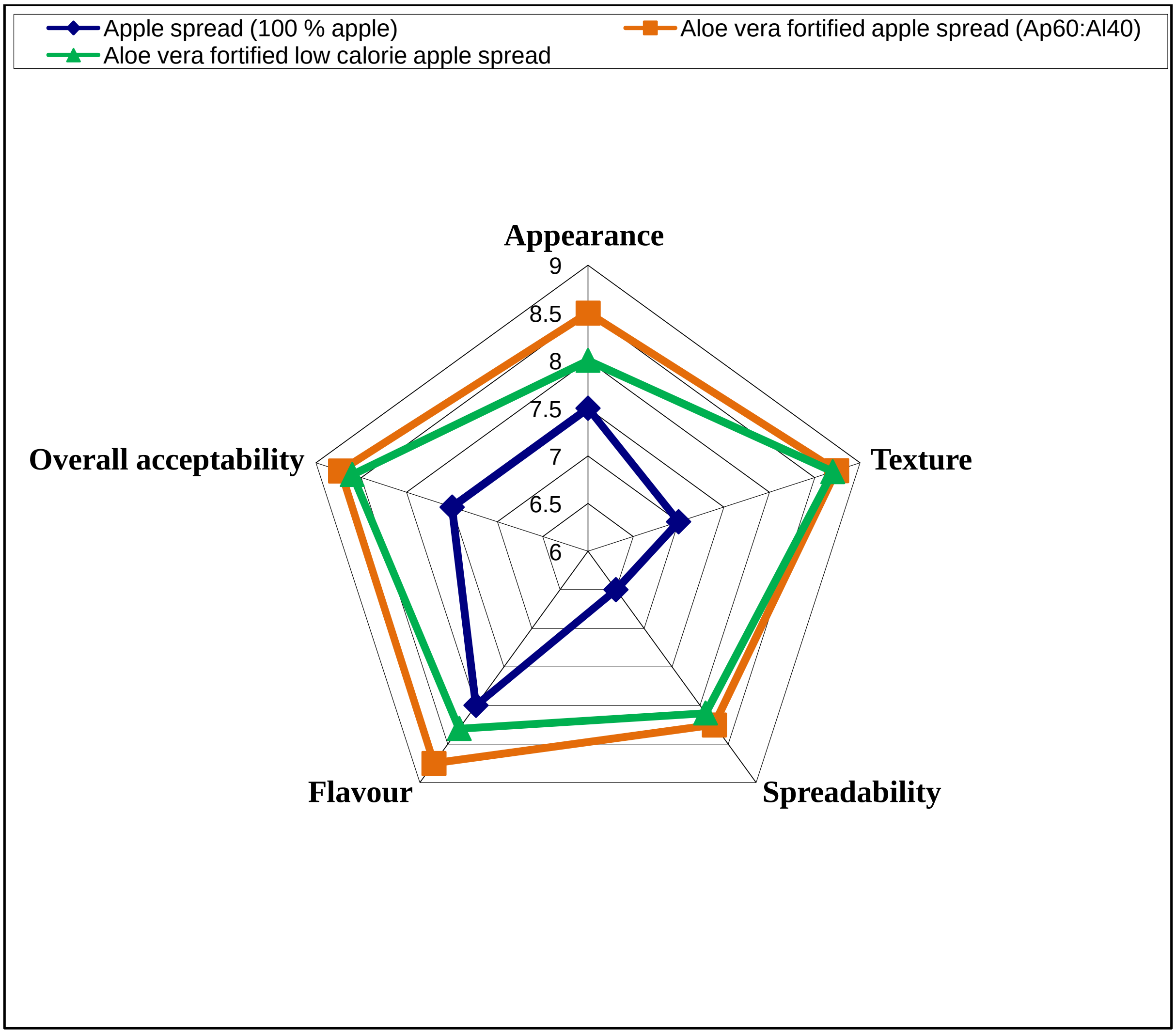


Figure 4.11: Comparison of sensory characteristics of apple spread and *Aloe vera* fortified apple spreads

4.6 CHANGES IN THE QUALITY ATTRIBUTES OF APPLE SPREADS DURING STORAGE

4.6.1 Changes in Physico-chemical Characteristics of Apple Spread, *Aloe vera* Fortified Apple Spread and *Aloe vera* Fortified Low Calorie Apple Spread

4.6.1.1 Moisture content

An appraisal of data in Table 4.10 reflects that there was a general decrease in moisture content of the spreads packed in glass jar. Whereas, the spread in polypropylene cups gained moisture during the storage intervals. The overall effect of treatments and storage intervals as shown by the data in interaction table signifies that the moisture content increased from 27.06 per cent to 28.98 per cent during storage. Among treatments, the mean moisture content ranged from 24.79 per cent and 30.45 per cent irrespective of packing material during storage period. On preparatory day, moisture content of 24.04 per cent was observed in treatment T_1 (apple spread), 27.39 per cent in treatment T_2 (*Aloe vera* fortified apple spread) and 29.76 per cent in treatment T_3 (low calorie apple spread) which decreased to 21.44, 25.41 and 27.73 per cent in the glass jars, while it increased to 30.35, 33.09 and 35.81 per cent in the polypropylene cups, respectively after six months of storage. The possible reason for increased level of moisture content in polypropylene cups might be due to the permeability of plastic caps. Whereas, glass jars are inert and air tight packaging material and not allow moisture to enter inside the product. The higher water content of low calorie spread might be due to the hydrocolloids present in the formulation, which contribute to higher water retention in the product (Toneli *et al*., 2005). The findings for moisture content are in accordance with the results of Istrati *et al.* (2013) in goji fruit jams and jellies. The decrease in moisture content in glass jar might be due to the increase in total soluble solids and total sugars that binds free water in the spread.

The overall effect of packaging material (P) and treatments were found to be significant, while the storage intervals (I) were found to be non-significant. However, combined effect of packaging material and storage interval (P×I); packaging material and treatment (P×T); treatment and storage interval (T×I); treatment, storage interval and packaging material (T×P×I) was observed as non-significant.

Table 4.10: Effect of treatment and packaging material on moisture content (%) of apple spreads during storage

P	**Glass jars**			**Mean**	**Polypropylene cups**			**Mean**
I	**Months**				**Months**			
T	**0**	**3**	**6**		**0**	**3**	**6**	
T_1	24.04	22.71	21.44	**22.73**	24.04	26.15	30.35	**26.85**
T_2	27.39	26.03	25.41	**26.28**	27.39	29.70	33.09	**30.06**
T_3	29.76	28.39	27.73	**28.63**	29.76	31.22	35.81	**32.26**
Mean	**27.06**	**25.71**	**24.86**		**27.06**	**29.02**	**33.08**	
Mean (P)	**25.88**				**29.72**			

T×I interaction table					**$CD_{(0.05)}$**	
T	**0**	**3**	**6**	**Mean (T)**	Packaging material (P)	3.01
T_1	24.04	24.43	25.90	**24.79**	Storage interval (I)	NS
T_2	27.39	27.87	29.25	**28.17**	Treatment (T)	3.69
T_3	29.76	29.81	31.77	**30.45**	P×I	NS
Mean (I)	**27.06**	**27.37**	**28.98**		P×T	NS
					T×I	NS
					T×P×I	NS

T_1: Apple spread, T_2: *Aloe vera* fortified apple spread, T_3: Low calorie apple spread

4.6.1.2 TSS

Data pertaining to total soluble solids presented in Table 4.11 indicated a gradual increase in TSS of spread packed in glass jar while, gradual decrease in the TSS of spread packed in polypropylene cups. Increase in the TSS might be due to solubilization of carbohydrates and other constituents due to the presence of acid during storage as reported by Haq and Darakshan (2014). The overall effect of treatments and storage intervals as shown by the data in interaction table indicated that TSS decreased from 60.66 to 51.70 ºB after six months of storage. TSS 60.80 ºB was observed in treatment T_1 (apple spread), 60.60 ºB was observed in treatment T_2 (*Aloe vera* fortified apple spread) and 51.48 ºB in treatment T_3 (low calorie apple spread) which increased to 64.19, 63.73 and 55.74 ºB in the glass jars and decreased to 56.15, 55.64 and 47.80 ºB in the polypropylene cups, respectively after six months of storage at ambient temperature. Effect of packaging material (P) and treatment (T) was observed to be significant while effect of storage interval (I) was found to be non-significant. Further, the combined effect of packaging material and storage interval (P×I); treatment and storage interval (T×I); packaging material and treatment (P×T); treatment, storage interval and packaging material (T×I×P) was observed as non-significant.

Table 4.11: Effect of treatment and packaging material on TSS (ºB) of apple spreads during storage

P	Glass jars			Mean	Polypropylene cups			Mean
I / T	Months				Months			
	0	3	6		0	3	6	
T_1	60.80	62.94	64.19	**62.64**	60.80	59.08	56.15	**58.68**
T_2	60.60	61.74	63.73	**62.02**	60.60	58.26	55.64	**58.17**
T_3	51.48	53.38	55.74	**53.53**	51.48	50.32	47.80	**49.87**
Mean	**57.63**	**59.35**	**61.22**		**57.63**	**55.62**	**53.20**	
Mean (P)	**59.40**				**55.57**			

T×I interaction table					CD(0.05)	
T	**0**	**3**	**6**	**Mean (T)**	Packaging material (P)	1.54
T_1	60.80	61.01	60.17	**57.63**	Storage interval (I)	NS
T_2	60.60	60.00	59.69	**57.62**	Treatment (T)	1.88
T_3	51.48	51.85	51.77	**57.21**	P×I	NS
Mean (I)	**60.66**	**60.10**	**51.70**		P×T	NS
					T×I	NS
					T×P×I	NS

T_1: Apple spread, T_2: *Aloe vera* fortified apple spread, T_3: Low calorie apple spread

Decrease in TSS in the spread packed in polypropylene cup occurred possibly due to increase in the moisture content. While, increase in the TSS of apple spreads packed in glass jars might be due to hydrolysis of sugars in the presence of acid during storage (Ehsan *et al*., 2003; Shakir *et al.,* 2008; Hussain and Shakir, 2010). Isah (2017) observed an increase in total soluble solids of the developed locust bean fruit jam. Similar results were reported by Haq and Darakshan (2014) in the prepared apricot-date jam.

4.6.1.3 Reducing sugars

The addition of low calorie sweeteners i.e. sweetos affected the reducing sugar of *Aloe vera* fortified low calorie functional apple spread. Data in Table 4.12 revealed that the mean reducing sugars were maximum (26.25%) for spread packed in glass jars and minimum (23.78%) for spreads packed in the polypropylene cups. The initial reducing sugars content of 22.92, 24.19 and 29.10 per cent were recorded in apple spread, *Aloe vera* fortified apple spread and low calorie apple spread which increased up to 24.98, 26.15 and 30.65 per cent, respectively during sixth months of spreads packed in glass jars. On the other hand, reducing sugars decreased in corresponding spreads as 19.54, 20.75 and 26.02 per cent during sixth months when packed in polypropylene cups.

Table 4.12: Effect of treatment and packaging material on reducing sugars (%) of apple spreads during storage

P	**Glass jars**			**Mean**	**Polypropylene cups**			**Mean**
I	**Months**				**Months**			
T	**0**	**3**	**6**		**0**	**3**	**6**	
T_1	22.92	23.13	24.98	**23.68**	22.92	21.32	19.54	**21.26**
T_2	24.19	25.26	26.15	**25.20**	24.19	22.80	20.75	**22.58**
T_3	29.10	29.87	30.65	**29.87**	29.10	27.41	26.02	**27.51**
Mean	**25.40**	**26.09**	**27.26**		**25.40**	**23.84**	**22.10**	
Mean (P)	**26.25**				**23.78**			

T×I interaction table

T	**0**	**3**	**6**	**Mean (T)**
T_1	22.92	24.19	29.10	**22.47**
T_2	22.23	24.03	28.64	**23.89**
T_3	22.26	23.45	28.34	**28.70**
Mean (I)	**25.40**	**24.97**	**24.68**	

$CD_{(0.05)}$	
Packaging material (P)	NS
Storage interval (I)	NS
Treatment (T)	4.04
P×I	NS
P×T	NS
T×I	NS
T×P×I	NS

T_1: Apple spread, T_2: *Aloe vera* fortified apple spread, T_3: Low calorie apple spread

Further, among the treatments the maximum mean (28.70 per cent) value for reducing sugars was found to in T_3 while, minimum value (22.47 per cent) was in T_1. It was recorded that during entire storage period, the combined effect of the packaging material and storage interval (P×I); packaging material and treatment (P×T); treatment and storage interval (T×I); treatment, packaging material and storage interval (T×P×I) on reducing sugars of spreads were found to be non-significant.

The decrease of reducing sugar contents might be due to the contribution of the reducing sugars to non-enzymatic browning phenomenon and hydroxymethyl furfural formation (Pavlova *et al.,* 2013). While the increase in reducing sugars during storage may be due to inversion of non-reducing sugars to reducing sugars under acidic condition (Isah, 2017).

4.6.1.4 Total sugars

Total sugars in the spread (T_3) containing low-calorie sweetener in the place of sucrose were reported less as compared to spreads containing 100 per cent sucrose. The mean total sugars were found to increase from 52.19 per cent to 53.69 and decreased to 49.03 per cent in the spreads packed in the glass jars and polypropylene cups, respectively.

Table 4.13: Effect of treatment and packaging material on total sugars (%) of apple spread during storage

P	**Glass jars**			**Mean**	**Polypropylene cups**			**Mean**
T \ I	**Months**				**Months**			
	0	**3**	**6**		**0**	**3**	**6**	
T_1	55.83	56.21	57.37	**56.47**	55.83	54.95	52.64	**54.47**
T_2	58.66	59.09	59.96	**59.24**	58.66	57.12	55.79	**57.19**
T_3	42.07	42.89	43.74	**42.90**	42.07	41.52	38.66	**40.75**
Mean	**52.19**	**52.73**	**53.69**		**52.19**	**51.20**	**49.03**	
Mean (P)	**52.87**				**50.80**			

T×I interaction table					**$CD_{(0.05)}$**	
T	**0**	**3**	**6**	**Mean (T)**	Packaging material (P)	NS
T_1	55.83	55.58	55.01	**55.47**	Storage interval (I)	NS
T_2	58.66	58.11	57.88	**58.21**	Treatment (T)	3.87
T_3	42.07	57.88	41.21	**41.83**	P×I	NS
Mean (I)	**52.19**	**51.96**	**51.36**		P×T	NS
					T×I	NS
					T×P×I	NS

T_1: Apple spread, T_2: *Aloe vera* fortified apple spread, T_3: Low calorie apple spread

The overall effect of treatment (T) on total sugars of spread was observed to be significant and maximum and minimum mean values of total sugars found in T_2 (58.21 %) and T_3 (41.83 %), respectively. Table 4.13 revealed that the combined effect of the packaging material and storage interval (P×I); packaging material and treatment (P×T); treatment and storage interval (T×I); treatment, packaging material and storage interval (T×P×I) was found to be non-significant.

According to Kumar and Deen (2017), hydrolysis of polysaccharides like pectin and starch could be one the reason for increase in the sugar content. Similar observations were also observed by many workers like Deen and Singh (2013) in karonda jelly. Fungal growth during sixth month of the spreads in the polypropylene cups could lead to reduction in the total sugars as fungus consumes sugar for the growth purpose (Abedayo and Salam, 2017).

4.6.1.5 Titratable acidity

Data pertaining to titratable acidity presented in Table 4.14 revealed a significant increase in titratable acidity of spread during entire storage period of six months. The mean titratable acidity were found to increase from 0.57 to 0.64 and 1.06 per cent in spreads packed in glass jars and polypropylene cups, respectively. The overall effect of packaging material reflected the maximum mean value of 0.75 per cent for titratable acidity in spread packed in polypropylene cups and minimum value of 0.61 per cent in glass jar. The overall effects of treatment (T) on titratable acidity of spread were observed to be non-significant. During zero month of storage, the initial titratable acidity 0.54 per cent was recorded in treatment T_1, 0.57 per cent in treatment T_2 and 0.60 per cent in treatment T_3 which increased to 0.60, 0.64 and 0.69 per cent packed in glass jars and 0.91, 1.05 and 1.23 per cent packed in the polypropylene cups after six months of storage. Further, overall and combined effects of packaging material and storage intervals on titratable acidity were observed to be significant. On the other hand, overall and combined effect of packaging material, storage interval and treatment on the pH of apple spreads was found to be non-significant (Data not given).

The increase in acidity might be due to acid formation, degradation of polysaccharides and oxidation of reducing sugars or by break down of pectin in to pectenic acid. The other possible reason for the rise in acidity was that the concentration of weekly-ionized acid and their salts increased during storage, resulting in increased acidity. These results are in accordance with the findings of Hussain and Shakir (2010) in the different samples of apple and apricot mixed fruit jam. Kanwal *et al.* (2017) and Rahman (2018) have also recorded a significant increase in titratable acidity of the guava jam and strawberry jam, respectively.

Table 4.14: Effect of treatment and packaging material on titratable acidity (%) of apple spreads during storage

P	Glass jars			Mean	Polypropylene cups			Mean
I / T	Months				Months			
	0	3	6		0	3	6	
T_1	0.54	0.58	0.60	**0.57**	0.54	0.61	0.91	**0.57**
T_2	0.57	0.61	0.64	**0.61**	0.57	0.63	1.05	**0.63**
T_3	0.60	0.63	0.69	**0.64**	0.60	0.65	1.23	**1.06**
Mean	**0.57**	**0.61**	**0.64**		**0.57**	**0.63**	**1.06**	
Mean (P)	**0.61**				**0.75**			

T×I interaction table					$CD_{(0.05)}$	
T	**0**	**3**	**6**	**Mean (T)**	Packaging material (P)	0.10
T_1	0.54	0.60	0.76	**0.63**	Storage interval (I)	1.13
T_2	0.57	0.62	0.85	**0.68**	Treatment (T)	NS
T_3	0.60	0.64	0.96	**0.73**	P×I	1.18
Mean (I)	**0.57**	**0.62**	**0.85**		P×T	NS
					T×I	NS
					T×P×I	NS

T_1: Apple spread, T_2: *Aloe vera* fortified apple spread, T_3: Low calorie apple spread

4.6.1.6 Ascorbic acid

It is apparent from the data in Table 4.15 that there was a significant decrease in ascorbic acid of apple spread during six months of storage period. Decrease in ascorbic acid content was found to be more prominent in spread packed in polypropylene cups as compared to spread packed in glass jars. On the preparation day, ascorbic acid content 14.23 mg/100g was observed in treatment T_1 (apple spread), 12.39 mg/100g in T_2 (*Aloe vera* fortified apple spread) and 13.05 mg/100g in T_3 (low calorie apple spread) which decreased to 9.53, 7.04 and 7.77 mg/100g when packed in glass jars and 6.56, 7.15, 5.38 mg/100g when packed in polypropylene cups, respectively after six months of storage. The mean ascorbic acid was found to decrease from 13.22 mg/100g to 8.11 mg/100g and 6.36 mg/100g in the spreads packed in glass jars and polypropylene cups, respectively. The overall effect of packaging material (P) on ascorbic acid was found to be non-significant.

According to Klopotek *et al.* (2005) and Rahman (2018), ascorbic acid content is unstable to heat and oxygen which is oxidized to non antioxidant effective substances. The decrease in ascorbic acid was due to prolong storage at high temperature. In addition, fermentation of the product also reduced the ascorbic acid content because enzymes and oxygen are capable of attacking and inactivating ascorbic acid (Mankar *et al.,* 2015).

Table 4.15: Effect of treatment and packaging material on ascorbic acid content (mg/100g) of apple spreads during storage

P	**Glass jars**			**Mean**	**Polypropylene cups**			**Mean**
T \ **I**	**Months**				**Months**			
	0	**3**	**6**		**0**	**3**	**6**	
T_1	14.23	12.80	9.53	**12.19**	14.23	11.62	6.56	**10.80**
T_2	12.39	10.66	7.04	**10.03**	12.39	9.65	7.15	**9.73**
T_3	13.05	9.76	7.77	**10.19**	13.05	9.83	5.38	**9.42**
Mean	**13.22**	**11.07**	**8.11**		**13.22**	**10.37**	**6.36**	
Mean (P)	**10.80**				**9.98**			

T×I interaction table					**$CD_{(0.05)}$**	
T	**0**	**3**	**6**	**Mean (T)**	Packaging material (P)	NS
T_1	14.23	12.21	8.05	**11.50**	Storage interval (I)	1.05
T_2	12.39	10.16	7.10	**9.88**	Treatment (T)	1.05
T_3	13.05	9.80	6.58	**9.81**	P×I	NS
Mean (I)	**13.22**	**10.72**	**7.24**		P×T	NS
					T×I	NS
					T×P×I	NS

T_1: Apple spread, T_2: *Aloe vera* fortified apple spread, T_3: Low calorie apple spread

4.6.1.7 Total phenols

There was a significant decrease in total phenols among different treatments of apple spreads during storage as shown in Table 4.16. Among both the packaging materials, the mean total phenols were observed as 55.45 and 52.79 mg/100g in spreads packed in the glass jars and polypropylene cups, respectively. The overall effect of treatments and storage intervals as depicted by the data in interaction table signifies that the total phenols decreased from 62.91 to 46.23 mg/100g with increase in storage intervals. Further, the overall effect of packaging material on total phenols was observed to be non-significant. The combined effect of treatment, packaging material and storage interval on ascorbic acid was recorded to be non-significant. Our results for total phenolics are obtained in accordance with Rababah *et al.* (2011) who found decrease in phenolics during storage in strawberry jam. The reduction of total phenolics during jam cooking might be due to disruption of the cell structure during longer storage. Another possible reason might be due to phenols involvement in the formation of polymeric compounds by complexion with protein and their subsequent precipitations. These results are in well coherence with Sharma *et al.* (2019) and Sharma *et al.* (2020) in herbal apple-whey RTS beverage and low calorie apple-whey based RTS beverage, respectively.

Table 4.16: Effect of treatment and packaging material on total phenols (mg/100g) of apple spreads during storage

P	Glass jars			Mean	Polypropylene cups			Mean
I / T	Months				Months			
	0	3	6		0	3	6	
T_1	36.69	30.74	24.47	**30.63**	36.69	28.08	20.68	**28.48**
T_2	77.27	70.78	65.71	**71.25**	77.27	67.90	59.48	**68.22**
T_3	74.66	63.28	55.32	**64.45**	74.66	58.57	51.72	**61.68**
Mean	**62.91**	**54.93**	**48.50**		**62.91**	**51.52**	**43.96**	
Mean (P)	**55.45**				**52.79**			

T×I interaction table					CD(0.05)	
T	**0**	**3**	**6**	**Mean (T)**		
					Packaging material (P)	NS
T_1	36.69	29.41	22.58	**29.56**	Storage interval (I)	3.29
T_2	77.27	69.34	62.60	**69.74**	Treatment (T)	3.29
T_3	74.66	60.34	53.52	**63.07**	P×I	NS
Mean (I)	**62.91**	**53.23**	**46.23**		P×T	NS
					T×I	NS
					T×P×I	NS

T_1: Apple spread, T_2: *Aloe vera* fortified apple spread, T_3: Low calorie apple spread

4.6.1.8 Antioxidant potential

Data presented in Table 4.17 revealed that the antioxidant potential of spreads experienced as significant decrease during storage intervals in both the packaging materials. The overall effect of packaging material reflected that the mean maximum antioxidant potential was recorded in glass jars (41.45 %) and minimum in polypropylene cups (39.37 %). Further, the antioxidant potential was found to decrease from an initial mean value of 46.88 to 35.56 per cent and 31.30 per cent in spreads packed in glass jar and polypropylene cups, respectively during storage period of six months. The overall effect of treatments and storage intervals as shown by the data in interaction table indicates that antioxidant potential decreased from 46.88 to 33.43 per cent during six months of storage. Further, combined effect of packaging material and storage intervals (P×I); packaging material and treatments (P×T); treatments and storage intervals (T×I); treatment, packaging material and storage interval (T×P×I) on ascorbic acid content were found to be non-significant.

According to Abolila *et al.* (2015) and Vukoja *et al.* (2019), the decrease in antioxidants can be attributed to the destruction of phytochemicals, phenolics compounds as well as ascorbic acid as a result of thermal treatment. Similar decreasing results were reported by Patras *et al.* (2011) in strawberry jam and Sharma and Thakur (2017) in bittergourd- aonla blended squash.

Table 4.17: Effect of treatment and packaging material on antioxidant potential (% free radical scavenging activity) of apple spreads during storage

P	Glass jars			Mean	Polypropylene cups			Mean
T \ I	Months				Months			
	0	**3**	**6**		**0**	**3**	**6**	
T_1	31.51	25.50	18.59	**25.20**	31.51	22.27	14.46	**22.75**
T_2	55.46	50.66	44.09	**50.07**	55.46	49.82	40.24	**48.51**
T_3	53.67	49.59	43.99	**49.08**	53.67	47.69	39.21	**46.86**
Mean	**46.88**	**41.92**	**35.56**		**46.88**	**39.93**	**31.30**	
Mean (P)	**41.45**				**39.37**			

T×I interaction table

T	**0**	**3**	**6**	**Mean (T)**
T_1	31.51	23.89	16.53	**23.97**
T_2	55.46	50.24	42.17	**49.29**
T_3	53.67	48.64	41.60	**47.97**
Mean (I)	**46.88**	**40.92**	**33.43**	

$CD_{(0.05)}$	
Packaging material (P)	1.99
Storage interval (I)	2.44
Treatment (T)	2.44
P×I	NS
P×T	NS
T×I	NS
T×P×I	NS

T_1: Apple spread, T_2: *Aloe vera* fortified apple spread, T_3: Low calorie apple spread

4.6.1.9 Water activity

An appraisal of data in Table 4.18 reflects that there was a general decrease in water activity of the spreads packed in glass jar. Whereas, the spread packed in polypropylene cups had increasing trend in water activity during entire storage period. The overall effect of treatments and storage intervals as shown by the data in interaction table indicated that the water activity increased from 0.78 to 0.80 during zero to sixth months of storage. The overall effect of packaging material reflected the maximum mean value of 0.82 for water activity in spreads packed in polypropylene cups and minimum of 0.76 in glass jars. The interactive effect of storage interval (I) and treatment (T) was found to be non-significant. Whereas, a non-significant difference was observed for combined effect of packaging material, storage interval and treatment on water activity. The decrease in water activity during storage might be due to association of sugars and water through hydrogen bonding. While, the increase in water activity in polypropylene cups might be due to increase in moisture content of the product. Menezes *et al.* (2011) and Kanwal *et al.* (2017) also reported similar decrease in water activity value of guava preserves and guava jam, respectively.

Table 4.18: Effect of treatment and packaging material on water activity of apple spreads during storage

P	Glass jars			Mean	Polypropylene cups			Mean
I / T	Months				Months			
	0	3	6		0	3	6	
T_1	0.75	0.74	0.72	**0.74**	0.75	0.77	0.83	**0.78**
T_2	0.78	0.77	0.75	**0.77**	0.78	0.81	0.86	**0.82**
T_3	0.81	0.79	0.76	**0.79**	0.81	0.84	0.89	**0.85**
Mean	**0.78**	**0.77**	**0.74**		**0.78**	**0.81**	**0.86**	
Mean (P)	**0.76**				**0.82**			

T×I interaction table					$CD_{(0.05)}$	
T	0	3	6	Mean (T)	Packaging material (P)	0.06
T_1	0.75	0.76	0.78	**0.76**	Storage interval (I)	NS
T_2	0.78	0.79	0.81	**0.79**	Treatments (T)	NS
T_3	0.81	0.82	0.83	**0.82**	P×I	NS
Mean (I)	**0.78**	**0.79**	**0.80**		P×T	NS
					T×I	NS
					T×P×I	NS

T_1: Apple spread, T_2: *Aloe vera* fortified apple spread, T_3: Low calorie apple spread

4.6.1.10 Non-enzymatic browning (NEB)

Non-enzymatic browning (NEB) values in spreads significantly increased during storage as revealed in Table 4.19. Among different packaging materials, the mean NEB was observed as same i.e. 0.45 in spread packed in glass jars and 0.47 in spread packed in polypropylene cups. The initial NEB, 0.45 was observed in T_1 (apple spread), 0.34 was observed in T_2 (*Aloe vera* fortified apple spread) and 0.26 was observed in T_3 (low calorie apple spread) which increased to 0.62, 0.54 and 0.48 in spreads packed in glass jars and 0.74, 0.58 and 0.40 in spread packed in polypropylene cups, respectively during six months of storage. Further, among the treatments the maximum mean value for reducing sugars was found in T_1 (0.57) while, minimum value was in T_3 (0.36).

A significant increase in NEB of spreads was recorded in different treatments during storage. Further, combined effect of packaging material and storage interval (P×I); packaging material and treatment (P×T); treatment and storage interval (T×I); treatment, packaging material and storage interval (T×P×I) on non-enzymatic browning (NEB) content were found to be non-significant. A significant increase in NEB during storage period might be due to formation of furfural and hydroxyl furfural by aerobic and anaerobic degradation of ascorbic acid and also due to reaction between sugars, ascorbic acid and organic acids. Similar increase in NEB was discussed by Deen and Singh (2013); Kumar and Deen (2017) in karonda jelly and guava jelly, respectively.

Table 4.19: Effect of treatment and packaging material on non-enzymatic browning (NEB) of apple spreads during storage

P	Glass jars			Mean	Polypropylene cups			Mean
I	Months				Months			
T	0	3	6		0	3	6	
T_1	0.45	0.56	0.62	**0.54**	0.45	0.59	0.74	**0.59**
T_2	0.34	0.45	0.54	**0.44**	0.34	0.49	0.58	**0.47**
T_3	0.26	0.35	0.48	**0.36**	0.26	0.41	0.40	**0.36**
Mean	**0.35**	**0.45**	**0.55**		**0.35**	**0.50**	**0.69**	
Mean (P)	**0.45**				**0.47**			

T×I interaction table					**$CD_{(0.05)}$**	
T	**0**	**3**	**6**	**Mean (T)**	Packaging material (P)	NS
T_1	0.45	0.58	0.68	**0.57**	Storage interval (I)	0.08
T_2	0.34	0.47	0.56	**0.46**	Treatment (T)	0.08
T_3	0.26	0.38	0.44	**0.36**	P×I	NS
Mean (I)	**0.35**	**0.48**	**0.56**		P×T	NS
					T×I	NS
					T×P×I	NS

T_1: Apple spread, T_2: *Aloe vera* fortified apple spread, T_3: Low calorie apple spread

4.6.1.11 Fat content

A perusal of data in Table 4.20 revealed that the fat content in spreads experienced a significant effect of storage interval and treatment. The overall effect of treatment and storage interval clearly depicted that the fat content decreased from 1.75 to 1.20 per cent with the increase in storage interval. While, overall effect of packaging material (P) on fat content of spread was found to be non-significant. The mean fat content was found to decrease from 1.75 to 1.36 and 1.04 per cent in the spreads packed in glass jars and polypropylene cups, respectively. The mean maximum (1.56 %) fat content was found in the spreads packed in glass jars while minimum (1.36 %) in polypropylene cups packed spreads.

Data further revealed that the combined effects of packaging material and storage intervals (P×I); packaging material and treatments (P×T); treatments and storage intervals (T×I); treatment, packaging material and storage interval (T×P×I) on fat content were found to be non-significant. The decrease in fat content of spread during storage might be due to hydrolysis of triglycerides and increase in moisture content. The results are in accordance with Chauhan *et al*. (2012) and Shahanas *et al.* (2019) who has done storage study of coconut jam. Similarly, Pavlova *et al.* (2013) observed similar decreasing trend of the fat content in raspberry and peach mixed fruit jam during storage.

Table 4.20: Effect of treatment and packaging material on fat content (%) of apple spreads during storage

P	Glass jars			Mean	Polypropylene cups			Mean
I / T	Months				Months			
	0	3	6		0	3	6	
T_1	2.28	1.97	1.62	**1.96**	2.28	1.61	1.55	**1.81**
T_2	1.71	1.68	1.56	**1.65**	1.71	1.20	0.85	**1.25**
T_3	1.25	1.03	0.91	**1.06**	1.25	1.05	0.73	**1.01**
Mean	**1.75**	**1.56**	**1.36**		**1.75**	**1.29**	**1.04**	
Mean (P)	**1.56**				**1.36**			

T×I interaction table					**$CD_{(0.05)}$**	
T	**0**	**3**	**6**	**Mean (T)**	Packaging material (P)	NS
T_1	2.28	1.79	1.59	**1.89**	Storage interval (I)	0.39
T_2	1.71	1.44	1.21	**1.45**	Treatment (T)	0.39
T_3	1.25	1.04	0.82	**1.04**	P×I	NS
Mean (I)	**1.75**	**1.42**	**1.20**		P× T	NS
					T×I	NS
					T×P×I	NS

T_1: Apple spread, T_2: *Aloe vera* fortified apple spread, T_3: Low calorie apple spread

4.6.1.12 Protein content

A significant decrease in protein content of different treatments was noticed during a storage period of six months (Table 4.21). The mean protein content was found to decrease from 1.35 per cent to 0.84 per cent in glass jars and to 0.57 per cent in polypropylene cups, respectively. The overall effect of treatments and storage intervals as shown by the data in interaction table signifies that the protein content decreased from 1.35 to 0.71 per cent with increase in storage intervals from zero to six months. Further while comparing the overall effect of packaging material was found to be non-significant. Data further revealed that the combined effects of packaging material and storage interval (P×I); packaging material and treatment (P×T); treatment and storage interval (T×I); treatment, packaging material and storage interval (T×P×I) on protein content were found to be non-significant.

Decrease in proteins might be due to the intervention of amino acid in non-enzymatic browning process as Maillard reactions (Djaoudene and Louaileche, 2016). In addition, proteolysis may occur during storage due to biochemical reactions and exposure to air consequently causes oxidative damage to the final product. Similar findings were revealed by Touati *et al.* (2014) in apricot jam after storage for 2 months at 25 ºC.

Table 4.21: Effect of treatment and packaging material on protein content (%) of apple spreads during storage

P	**Glass jars**			**Mean**	**Polypropylene cups**			**Mean**
I / T	**Months**				**Months**			
	0	**3**	**6**		**0**	**3**	**6**	
T_1	1.93	1.48	1.35	**1.59**	1.93	1.23	0.60	**1.25**
T_2	1.03	0.97	0.64	**0.88**	1.03	0.84	0.71	**0.86**
T_3	1.08	0.78	0.53	**0.80**	1.08	0.68	0.41	**0.72**
Mean	**1.35**	**1.08**	**0.84**		**1.35**	**0.92**	**0.57**	
Mean (P)	**1.09**				**0.95**			

T×I interaction table					**$CD_{(0.05)}$**	
T	**0**	**3**	**6**	**Mean (T)**	Packaging material (P)	NS
T_1	1.93	1.36	0.98	**1.42**	Storage interval (I)	0.36
T_2	1.03	0.91	0.68	**0.87**	Treatment (T)	0.36
T_3	1.08	0.73	0.47	**0.76**	P ×I	NS
Mean (I)	**1.35**	**1.00**	**0.71**		P ×T	NS
					T×I	NS
					T×P×I	NS

T_1: Apple spread, T_2: *Aloe vera* fortified apple spread, T_3: Low calorie functional apple spread

4.6.1.13 Ash content

The data in Table 4.22 represented a significant difference in the ash content of different treatments of apple spreads i.e. 2.16, 1.84 and 1.52 per cent. The overall effect of treatments (T) on ash content was found to be significant. While, the effects of packaging material (P) and storage intervals (I) were found to be non-significant. The mean ash content decreased from 1.84 to 1.64 and 1.28 per cent in the spreads packed in glass jars and polypropylene cups, respectively. The combined effects of packaging material and storage intervals (P×I); packaging material and treatments (P×T); treatments and storage intervals (T×I); treatment, packaging material and storage interval (T×P×I) on ash content were found to be non-significant.

This decrease in ash content might be due to increased activities of microorganisms in polypropylene cups by utilizing the minerals for growth which resulted in reduction of mineral content. Similar observations were reported by Ashaye and Adeleke (2009) who observed a decrease in ash content of rosella jam during storage. Sutwal *et al.* (2019) also observed the significant decrease in ash content of the low calorie apple jam sweetened with stevia during storage.

Table 4.22: Effect of treatment and packaging material on ash content (%) of apple spreads during storage

P	**Glass jars**			**Mean**	**Polypropylene cups**			**Mean**
I / T	**Months**				**Months**			
	0	**3**	**6**		**0**	**3**	**6**	
T_1	2.16	2.09	2.04	**2.10**	2.16	1.82	1.50	**1.83**
T_2	1.84	1.53	1.49	**1.62**	1.84	1.43	1.35	**1.54**
T_3	1.52	1.44	1.38	**1.45**	1.52	1.02	0.98	**1.17**
Mean	**1.84**	**1.69**	**1.64**		**1.84**	**1.42**	**1.28**	
Mean (P)	**1.72**				**1.51**			
T×I interaction table					**$CD_{(0.05)}$**			
T	**0**	**3**	**6**	**Mean (T)**	Packaging material (P)			NS
T_1	2.16	1.96	1.77	**1.96**	Storage interval (I)			NS
T_2	1.84	1.48	1.42	**1.58**	Treatment (T)			0.50
T_3	1.52	1.23	1.18	**1.31**	P ×I			NS
Mean (I)	**1.84**	**1.56**	**1.46**		P ×T			NS
					T×I			NS
					T×P×I			NS

T_1: Apple spread, T_2: *Aloe vera* fortified apple spread, T_3: Low calorie functional apple spread

4.6.1.14 Energy value

Energy value of the spreads was estimated by using bomb calorimeter at different storage intervals and expressed as Kcal/100g. The data presented in Figure 4.12, the energy values of the spreads increased in sucrose containing apple spreads and decreased in low calorie apple spread. The initial energy value of 306.6 Kcal/100g was recorded in T_1 (apple spread), 291.63 Kcal/100g in T_2 (*Aloe vera* fortified apple spread) and 100.27 Kcal/100g in T_3 (*Aloe vera* fortified low calorie apple spread) which was increased to 314.18 Kcal/100g in T_1 and 300.20 Kcal/100g in T_2 while decreased in T_3 i.e. 95.01 Kcal/100g in glass jars during six months of storage. In this study, approximately 65 per cent reduction in calories observed in low calorie apple spread as compared to control sample. The reduced energy value in sweetos sweetened low calorie apple spread might be due to the fact that low calorie sweeteners provides very less calories (Sharma and Joshi, 2015; Sharma *et al.*, 2020). Naeem *et al.* (2015) has reported similar increase in energy value in apple jam sweetened with sucrose. Further, Tobal and Rodrigues (2019) observed the decrease in calorie value of low calorie pitana jam after 320 days of storage.

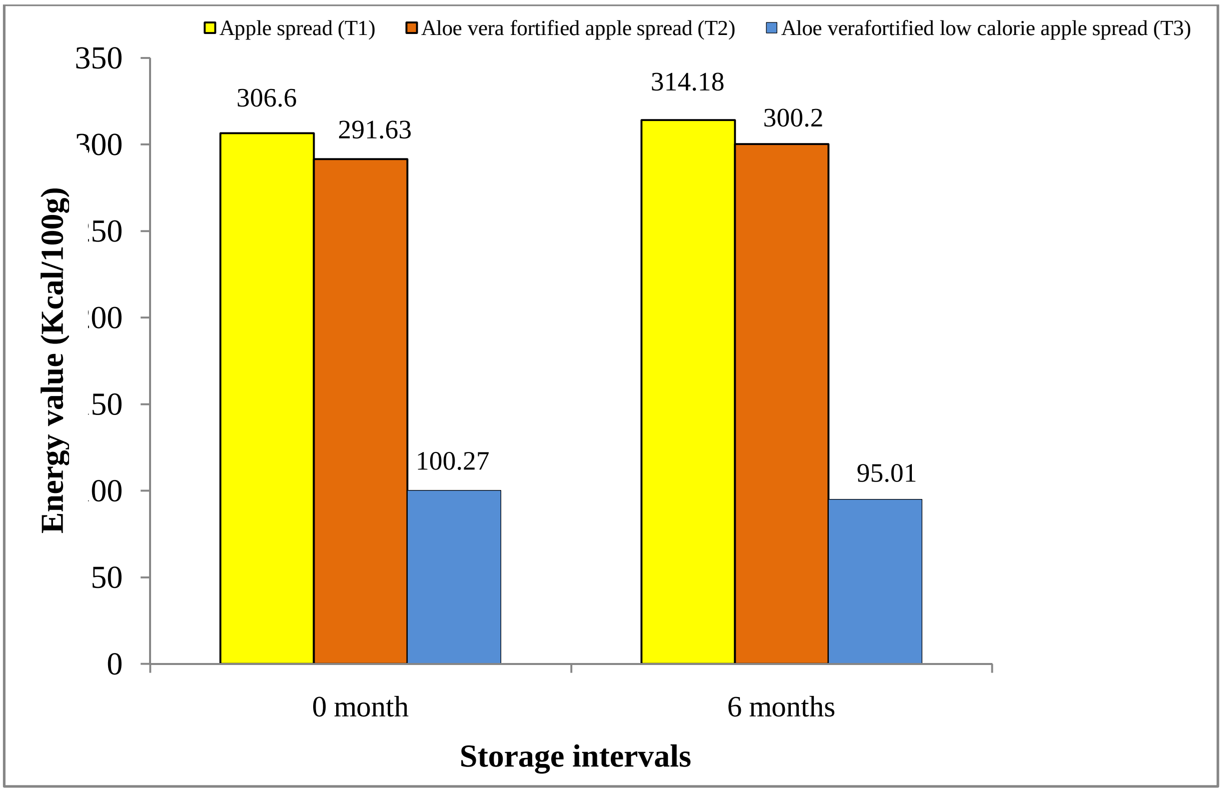


Figure 4.12: Energy value (Kcal/100g) of apple spread and *Aloe vera* fortified apple spreads

4.6.2 Changes in Sensory Characteristics of Apple Spread, *Aloe vera* Fortified Apple Spread and *Aloe vera* Fortified Low Calorie Apple Spread

Changes in sensory attributes of the apple spread, *Aloe vera* fortified apple spread and *Aloe vera* fortified low calorie functional apple spreads packed separately in the glass jars and polypropylene cups for storage intervals of 0, 3, 6 months were evaluated for various sensory characteristics *viz*. appearance, texture, spreadability, flavour and overall acceptability. The results obtained are presented in Table 23-27 and discussed below under as:

4.6.2.1 Appearance

The effect of packaging material and treatment on appearance score of apple spreads during storage is given in Table 4.23, which revealed a decrease in the appearance score of the spreads during six months of storage. Among different packaging material, the mean maximum score was recorded in spread packed in glass jars (7.77) and minimum for polypropylene cups (6.77). The overall effect of treatments and storage intervals as shown by the data in interaction table signifies that the appearance score decreased from an initial mean value of 8.00 to 6.27 after six months. However, appearance score was higher in glass jars compared to polypropylene cups, respectively. The combined effect of packaging material and storage intervals (P×I) indicates that during entire storage period the appearance score decreased from 8.00 to 7.57 in glass jars and 8.00 to 4.97 in polypropylene cups, respectively. However, the combined effects of packaging material and treatment (P×T); treatment and storage interval (T×I); treatment, packaging material and storage interval (T×P×I) on appearance score were found to be non-significant.

Table 4.23: Effect of treatment and packaging material on appearance score of apple spreads during storage

P	Glass jars			Mean	Polypropylene cups			Mean
I / T	Months				Months			
	0	3	6		0	3	6	
T_1	7.50	7.25	7.10	**7.28**	7.50	7.00	4.50	**6.33**
T_2	8.50	8.20	8.00	**8.23**	8.50	7.75	5.75	**7.33**
T_3	8.00	7.75	7.60	**7.78**	8.00	7.30	4.66	**6.65**
Mean	**8.00**	**7.73**	**7.57**		**8.00**	**7.35**	**4.97**	
Mean (P)	**7.77**				**6.77**			

T×I interaction table					**CD(0.05)**	
T	**0**	**3**	**6**	**Mean (T)**	Packaging material (P)	0.43
T_1	7.50	7.13	5.80	**6.81**	Storage interval (I)	0.52
T_2	8.50	7.98	6.87	**7.78**	Treatment (T)	0.52
T_3	8.00	7.53	6.13	**7.22**	P×I	0.74
Mean (I)	**8.00**	**7.54**	**6.27**		P×T	NS
					T×I	NS
					T×P×I	NS

T_1: Apple spread, T_2: *Aloe vera* fortified apple spread, T_3: Low calorie apple spread, Appearance score recorded on 9-point Hedonic scale

Lack of glossiness was found in sample T_3 (*Aloe vera* fortified low calorie apple spread) due to the absence of sucrose. Decrease in appearance during storage might be due to browning reactions, degradation of ascorbic content, polymerization of colour pigments and phenolic compounds. Similar results have been reported by Bhople *et al.* (2016) who observed decreasing trend in appearance of beetroot jam during storage (Sutwal *et al.*, 2019).

4.6.2.2 Texture

The data in Table 4.24 for texture score of apple spread indicated a significant difference among various treatments i.e. 7.00, 8.74 and 8.70. The overall effect of treatments and storage intervals as shown by the data in interaction table revealed that the texture score decreased from 8.15 to 6.93 with increase in storage interval from zero to six months. While, comparing with the effect of packaging material (P), texture score retained higher in glass jars as compared to polypropylene cups in different spreads.

Table 4.24: Effect of treatment and packaging material on texture score of apple spreads during storage

P	Glass jars			Mean	Polypropylene cups			Mean
I / T	Months				Months			
	0	3	6		0	3	6	
T_1	7.00	6.78	6.65	**6.81**	7.00	6.48	5.75	**6.41**
T_2	8.74	8.40	8.10	**8.41**	8.74	8.10	6.50	**7.78**
T_3	8.70	8.35	8.22	**8.42**	8.70	8.05	6.35	**7.70**
Mean	**8.15**	**7.84**	**7.66**		**8.15**	**7.54**	**6.20**	
Mean (P)	**7.88**				**7.30**			

T×I interaction table					CD(0.05)	
T	**0**	**3**	**6**	**Mean (T)**	Packaging material (P)	0.31
T_1	7.00	6.63	6.20	**6.61**	Storage interval (I)	0.39
T_2	8.74	8.25	7.30	**8.10**	Treatment (T)	0.39
T_3	8.70	8.20	7.29	**8.06**	P×I	0.54
Mean (I)	**8.15**	**7.69**	**6.93**		P×T	NS
					T×I	NS
					T×P×I	NS

T_1: Apple spread, T_2: *Aloe vera* fortified apple spread, T_3: Low calorie apple spread, Texture score recorded on 9-point Hedonic scale

The combined effect of packaging material and storage intervals (P×I) indicates that during entire storage period the texture score decreased from 8.15 to 7.66 in spread packed in glass jars and 6.20 in spread packed in polypropylene cups, respectively. Whereas, the combined effects of packaging material and treatments (P×T); treatments and storage intervals (T×I); treatment, packaging material and storage interval (T×P×I) on texture score were found to be non-significant.

Decrease in texture score during storage might be due to interaction between phenolics, degradation of colloidal particles and protein as well as the formation of complexes with pectin and phenolic compounds (Abolila *et al*., 2015; Sutwal *et al*., 2019). Our results are in conformity with the results discussed by Ullah *et al.* (2018) in the apple and carrot blended jams. Kanwal *et al.* (2017) and Rahman *et al.* (2018) reported similar findings in mango jam and guava jam, respectively.

4.6.2.3 Spreadability

The effect of packaging material and treatment on spreadability score of apple spread is given in the Table 4.25 a significant decrease in the scores was observed throughout the storage period of six months.

Table 4.25: Effect of treatment and packaging material on spreadability score of apple spreads during storage

P	**Glass jars**			**Mean**	**Polypropylene cups**			**Mean**
I	**Months**				**Months**			
T	**0**	**3**	**6**		**0**	**3**	**6**	
T_1	6.50	7.10	7.00	**6.36**	6.50	7.00	5.30	**6.07**
T_2	8.25	8.18	8.10	**8.18**	8.25	8.00	6.90	**7.72**
T_3	8.10	8.00	7.90	**7.93**	8.10	7.80	6.75	**7.55**
Mean	**7.62**	**7.54**	**7.32**		**7.62**	**7.40**	**6.32**	
Mean (P)	**7.49**				**7.11**			

T×I interaction table					**CD$_{(0.05)}$**	
T	**0**	**3**	**6**	**Mean (T)**		
					Packaging material (P)	0.38
T_1	6.50	6.42	5.73	**6.22**	Storage interval (I)	0.46
T_2	8.25	8.09	7.50	**7.95**	Treatment (T)	0.46
T_3	8.10	7.90	7.22	**7.74**	P×I	NS
Mean (I)	**7.62**	**7.47**	**6.82**		P×T	NS
					T×I	NS
					T×P×I	NS

T_1: Apple spread, T_2: *Aloe vera* fortified apple spread, T_3: Low calorie apple spread, spreadability score recorded on 9-point Hedonic scale

An average decrease in mean spreadability score of different treatments was revealed from initial value of 7.62 to 7.32 and 6.32 in glass jars and polypropylene cups, respectively. Among different packaging material the mean maximum score for spreadability was observed in glass jars (7.49) and minimum score in polypropylene cups (7.11). However, combined effects of packaging material and storage intervals (P×I); packaging material and treatments (P×T); treatments and storage intervals (T×I); treatment, packaging material and storage interval (T×P×I) on spreadability score were found to be non-significant.

Decrease in spreadability score might be due to hydrolysis of pectin during storage. The role of pectin is to form a network and affects its smooth texture (Rathore *et al.*, 2007). Decreasing trend in spreadability score was in evident with the sensory score recorded by Kanwal *et al.* (2017). Similarly, Muhammad *et al*. (2008) has reported the similar decreasing tend in the spreadability of apple jam during storage.

4.6.2.4 Flavour

Data presented in Table 4.26 showed a general decreasing trend of flavour score during storage. The overall effect of treatment (T) and storage interval (I) on flavour score of spread signifies that it decreased from 8.35 to 5.94. Among both the packaging material the mean maximum score was observed in glass jars (7.87) and minimum in polypropylene cups (6.68). The overall effect of treatments (T) was found to be non-significant. The combined effect of packaging material and storage intervals (P×I) on flavour signifies that during entire storage period, the flavour score decreased from 8.35 to 7.50 in spreads packed in glass jars and 4.38 in spreads packed in polypropylene cups, respectively. While, combined effects of packaging material and treatment (P×T); treatment and storage interval (T×I); treatment, packaging material and storage interval (T×P×I) on flavour score were found to be non-significant.

Very less scores were given by the panelists during sixth months of spreads packed in polypropylene cups on the basis of bad odor of the product because of which the product was not even tasted by them. Decline in the flavour score might be due to the fluctuation in the acidity of the spread (Rahman *et al.,* 2018). Another possible reason might be due to oxidative changes of sugars, enzymatic degradation of phenols and the loss of high volatile aromatic compound at high storage temperature (Sutwal *et al.,* 2019). Ozdogan and Yilmaz

(2011) and Patel and Naik (2013) recorded the similar flavour score in tomato jam and banana- pine apple blended jam, respectively during 90 days of storage.

Table 4.26: Effect of treatment and packaging material on flavour score of apple spreads during storage

P	Glass jars			Mean	Polypropylene cups			Mean
I \ T	Months				Months			
	0	3	6		0	3	6	
T_1	8.00	7.77	7.41	**7.73**	8.00	7.04	4.00	**6.35**
T_2	8.75	7.50	7.24	**7.83**	8.75	7.24	4.65	**6.88**
T_3	8.30	8.00	7.85	**8.05**	8.30	7.70	4.48	**6.83**
Mean	**8.35**	**7.76**	**7.50**		**8.35**	**7.33**	**4.38**	
Mean (P)	**7.87**				**6.68**			

T×I interaction table					$CD_{(0.05)}$	
T	0	3	6	Mean (T)	Packaging material (P)	0.47
T_1	8.00	7.41	5.71	**7.04**	Storage interval (I)	0.58
T_2	8.75	7.37	5.95	**7.36**	Treatment (T)	NS
T_3	8.30	7.85	6.17	**7.44**	P×I	0.82
Mean (I)	**8.35**	**7.54**	**5.94**		P ×T	NS
					T×I	NS
					T×P×I	NS

T_1: Apple spread, T_2: *Aloe vera* fortified apple spread, T_3: Low calorie apple spread, flavour score recorded on 9-point Hedonic scale

4.6.2.5 Overall acceptability

The data given in Table 4.27 clearly indicated a decrease in overall acceptability score of apple spreads during storage period. Among all the treatments decrease in overall acceptability score was found to be significant. The combined effect of packaging material and storage intervals (P×I) signified a decrease in overall acceptability score from 8.08 to 7.72 in spread packed in glass jars and 5.00 in spread packed in polypropylene cups, respectively. The mean value of treatments were observed to significantly different from each other i.e. T_1 (6.96), T_2 (7.98) and T_3 (7.34). While, combined effects of packaging material and treatment (P×T); treatment and storage interval (T×I); treatment, packaging material and storage interval (T×P×I) on flavour score were found to be non-significant. Decrease in overall acceptability during the storage period was due to the decrease in appearance, texture, spreadability and flavour score with increasing storage period. These results are in alignment with the study of Khan *et al.* (2015) in which a decreasing trend was observed in overall acceptability of apple jam from during storage. Similarly, Kinswe and Soesoe (2019) have also reported decrease in overall acceptability score of pineapple and mango mixed jam during storage.

Table 4.27: Effect of treatment and packaging material on overall acceptability score of apple spreads during storage

P	Glass jars			Mean	Polypropylene cups			Mean
I → Months / T ↓	0	3	6		0	3	6	
T_1	7.50	7.42	7.30	**7.41**	7.50	7.20	4.75	**6.52**
T_2	8.73	8.50	8.20	**8.48**	8.73	8.45	5.25	**7.48**
T_3	8.00	7.80	7.65	**7.82**	8.00	7.60	5.00	**6.87**
Mean	**8.08**	**7.91**	**7.72**		**8.08**	**7.78**	**5.00**	
Mean (P)	**7.90**				**6.95**			

T×I interaction table

T	0	3	6	Mean (T)
T_1	7.50	7.36	6.03	**6.96**
T_2	8.73	8.48	6.73	**7.98**
T_3	8.00	7.70	6.33	**7.34**
Mean (I)	**8.08**	**7.85**	**6.36**	

$CD_{(0.05)}$	
Packaging material (P)	0.26
Storage interval (I)	0.32
Treatment (T)	0.32
P×I	0.45
P×T	NS
T×I	NS
T×P×I	NS

T_1: Apple spread, T_2: *Aloe vera* fortified apple spread, T_3: Low calorie apple spread, Overall acceptability score recorded on 9-point Hedonic scale

4.7 MICROBIAL QUALITY OF APPLE SPREADS DURING STORAGE

Microbial analysis of apple spread, *Aloe vera* fortified apple spread and *Aloe vera* fortified low calorie functional apple spread in glass jars as well as polypropylene cups was performed during the storage intervals of 3 months and 6 months. A perusal of data presented in Table 4.28 reveals that, with the increase in storage intervals microbial load also increased. After third month of storage, microbial growth was started in polypropylene cups and no growth was observed in glass jars. After sixth months of storage, the microbial load in glass jars was observed under acceptable limit (not more than 40 count/g) given by FSSAI while microbial growth in polypropylene cups exceeded the acceptable limit.

In intermediate moisture foods most of the microbes are destroyed by heat processing and high sucrose concentration act as preservative which binds the water molecules and make it unavailable to microorganisms. But moisture transmission in the packaging material caused heavy bacterial and fungal growth in the product. Our results are in accordance with the results revealed by Vidhya and Narain (2011); Sutwal *et al.* (2017) in wood apple jam and low calorie apple jam. Whereas, Abedayo and Salam (2017) has reported the total fungal growth in the jams packed in transparent plastic cups and no growth in the jams packed in the glass jars during storage. Brandao *et al*. (2018) also discussed similar increase in microbial load in cerrado low calorie jam during 180 days of ambient storage.

Table 4.28: Effect of treatment and packaging material on microbial population (log cfu/mL) of apple spreads during storage

P	Glass jar		Polypropylene cup	
I	Months		Months	
T	3	6	3	6
T_1	No growth	0.8×10^4	0.9×10^4	5.6×10^4
T_2	No growth	0.4×10^4	0.5×10^4	4.5×10^4
T_3	No growth	0.5×10^4	0.6×10^4	4.9×10^4

cfu/mL = No. of colonies × dilution factor/volume of sample used (0.1mL)
Dilution factor = 1/dilution, cfu = colony forming units
T_1: Apple spread, T_2: *Aloe vera* fortified apple spread, T_3: Low calorie apple spread

4.8 COST OF PRODUCTION OF *ALOE VERA* FORTIFIED APPLE SPREADS

Cost incurred in preparation of apple spread, *Aloe vera* fortified apple spread and *Aloe vera* fortified low-calorie functional apple spread was calculated by keeping in view the inputs and the cost involved during processing. The comparative cost of production of above three spreads has been presented in the Table 4.29. The cost of production of apple spread was calculated on the basis of current prices of ingredients and nominal processing charges. The market price of sweetos (low calorie sweetener) was comparatively higher than sugar, therefore, the price of *Aloe vera* fortified low-calorie functional apple spread was calculated to higher as compared to other two spreads. The nominal processing charges, including cost of raw ingredients has been taken into consideration while calculating the cost of preparing products. The cost of apple spread was Rs 25.43/200g of glass jar and Rs 16.63/200g of spread packed in polypropylene cups. Whereas, cost of *Aloe vera* fortified apple spread packed in glass jar and polypropylene cups was recorded as Rs 27.51/200g and Rs 18.71/200g, respectively. It was concluded that the cost of *Aloe vera* fortified low calorie functional apple spread had the highest cost of production (48.81/200g glass jar and 40.01/200g polypropylene cup). However, cost of spreads packed in glass jars was comparatively higher because cost of empty jar was more as compared to that of polypropylene cups.

Table 4.29: Cost of production of apple spread, *Aloe vera* fortified apple spread and *Aloe vera* fortified low calorie apple spread

Particular		Rate (Rs.)	Apple spread		*Aloe vera* fortified apple spread		*Aloe vera* fortified low calorie apple spread	
			Quantity	Amount (Rs.)	Quantity	Amount (Rs.)	Quantity	Amount (Rs.)
Apple pulp		50.50/ Kg	4500g	227.25	2700g	136.4	2700g	136.4
Aloe vera pulp		102.96/ Kg	-	-	1800g	185.3	1800g	185.3
Sugar		44/ Kg	5500g	242	5500	242	-	-
Citric acid @ 0.5 %		600/ Kg	3g	1.8	3g	1.8g	3g	1.8g
Pectin @ 0.5 %		7000/Kg	5g	35	5g	35	5g	35
Sweetos		220/ Kg	-	-	-	-	5500	1210
Packaging material	Glass jars	12 / Jar	50	600	50	600	50	600
	Polypropylene cups	2/ Cup	100	200	100	200	100	200
Labels		1/label	50	50	50	50	50	50
Total cost of ingredients (Rs)	Glass jars	-	-	1156.05	-	1250.5	-	2218.5
	Polypropylene cups	-	-	756.05	-	850.5	-	1818.5
Processing cost @ 10 %	Glass jars	-	-	115.61	-	125.05	-	221.85
	Polypropylene cups	-	-	75.61	-	85.05	-	181.85
Total preparation cost (10 Kg)	Glass jars	-	-	1271.66	-	1375.55	-	2440.35
	Polypropylene cups	-	-	831.66	-	935.55	-	2000.35
Sale price per 200g glass jar/ polypropylene cups	Glass jars	-	-	25.43	-	27.51	-	**48.81**
	Polypropylene cups	-	-	16.63	-	18.71	-	**40.01**

Chapter-5

SUMMARY AND CONCLUSION

The present investigation entitled, **"Studies on development of *Aloe vera* fortified low calorie functional apple spread"** was performed in the Department of Food Science and Technology, Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (HP) during the year 2018-2020. For the fulfilment of present study, *Aloe vera* was procured from the Herbal Garden, Department of Forest Products, Dr Yashwant Singh University of Horticulture and Forestry, Nauni, Solan (HP) whereas, apple fruits (Golden Delicious) and other ingredients were procured from the local market of Solan and then brought to the Department of Food Science and Technology where pulp extraction and their preservation was done. Different experiments were carried out for the development of *Aloe vera* fortified apple spread by optimizing different combinations of apple pulp, *Aloe vera* pulp/gel, TSS, acidity and pectin using RSM. The best rated combination of *Aloe vera* fortified apple spread was further used for the preparation of *Aloe vera* fortified low calorie functional apple spread. Sucrose present in the optimized product was replaced by sweetos (fructooligosaccharide + sucralose) with equi-sweetness proportions (sucrose equivalent) of 0, 25, 50, 75 and 100 per cent. Both the products along with control sample were packed in glass jars and polypropylene cups, which were stored until used for quality analysis during storage at periodic intervals of 0, 3 and 6 months at ambient temperature (Appendix-II). Cost of production of control sample and best rated products were also calculated. The results obtained in the present study have been summarized and concluded briefly as under:

5.1 PHYSICO-CHEMICAL CHARACTERISTICS OF FRESH APPLE FRUIT AND *ALOE VERA* LEAVES

5.1.1 Apple fruit used in the present study weighed 127.03 g with a mean volume of 169.16 cc per fruit and size attributes of 66.91 mm length and 65.11 mm diameter, respectively. The edible portion and pulp yield of fruit was 88.5 per cent and 79.20 per cent. The visual colour of apple fruit was Yellow-Green 145 group (145-B). The fruits contained 85.24 per cent moisture content, 13.13 ºB total soluble solids, 0.46 per cent titratable acidity (as % malic acid), 16.70 mg/100g ascorbic acid, 7.35 per cent reducing sugar and 12.39 per cent total sugars with pH value of 3.44. The phenolic content and antioxidant activity of the fruits were observed as 66.14

mg/100g and 58.63 per cent, respectively. The parameters recorded were considered optimum for developing apple spread.

5.1.2 The various physico-chemical characteristics of *Aloe vera* leaves used in the present study were recorded as leaf weight (244.93 g), length (265.44 mm), diameter (79.71 mm), moisture content (98.27 %), edible portion (66.39 %), pulp yield (48.59 %), total soluble solids (2.04 ºB), titratable acidity (0.22 %), pH (4.54), ascorbic acid (10.07 mg/100g), reducing sugars (0.61 %) and total sugars (1.21 %). Whereas, the phenolic contents and antioxidant activity were recorded as 217.53 mg/100g and 82.98 per cent, respectively. Therefore, on the basis of physico-chemical attributes, the processed pulp/gel was found suitable for the development of *Aloe vera* fortified apple spreads.

5.2 PHYSICO-CHEMICAL CHARACTERISTICS OF FRESH APPLE PULP AND *ALOE VERA* PULP

5.2.1 The fresh apple pulp contained 13.62 ºB total soluble solids, 0.49 per cent titratable acidity (% malic acid), 3.39 pH, 10.50 mg/100g ascorbic acid, 7.48 per cent reducing sugar and 12.64 per cent total sugars. The total phenols and antioxidant potential of apple pulp were recorded as 40.30 mg/100g and 34.48 per cent, respectively. Apple pulp contained major minerals such as potassium (0.162 g/100g), phosphorus (0.480 g/100g), calcium (40 mg/100g), magnesium (3.21 mg/100g) and minor minerals such as copper (0.43 mg/100g), iron (1.22 mg/100g) and manganese (0.70 mg/100g).

5.2.2 After processing, *Aloe vera* gel/pulp contained 2.16 ºB total soluble solids, 0.30 per cent titratable acidity (% citric acid), 6.35 mg/100g ascorbic acid, 0.64 per cent reducing sugars and 1.41 per cent total sugar with pH of 4.47. Total phenols and antioxidant potential of *Aloe vera* gel were recorded as 185.53 mg/100g and 68.21 per cent, respectively. Further, different minerals such as potassium (0.165 g/100g), phosphorus (0.491 g/100g), calcium (11.50 mg/100g), copper (0.70 mg/100g), iron (0.67 mg/100g), manganese (1.60 mg/100g) and magnesium (3.23 mg/100g) were also presented in the fresh extracted *Aloe vera* gel.

5.3 APPLE SPREAD, *ALOE VERA* FORTIFIED APPLE SPREAD AND *ALOE VERA* FORTIFIED LOW CALORIE APPLE SPREAD

5.3.1 The control sample of apple spread was developed by using 100 per cent apple pulp with 60 ºB TSS, 0.5 per cent acidity and 0.5 per cent level of pectin. The prepared apple spread had TSS of 60.8 ºB, 0.54 per cent titratable acidity and 0.75 water activity with 24.04 per cent of moisture content. The reducing sugars, total sugars, NEB, protein, fat, ascorbic acid, total phenols and antioxidants potential were observed as 22.92 per cent, 55.83 per cent, 0.45, 2.16 per cent, 2.28 per cent, 14.23 mg/100g, 36.69 mg/100g and 31.51 per cent, respectively.

5.3.2 Different combinations of *Aloe vera* pulp, TSS, acidity and pectin were designed by Response Surface Methodology for optimizing best product on the basis of different responses. It was observed that, all the polynomial models were well fitted as the coefficient of determination (R^2) and adequate precision were more than 80 per cent and 4, respectively. While, the 3-D plot graphs and equations explained the linear, quadratic and interactive effects of the responses. Further, a confirmatory test in which the predicted and observed values were compared by paired t-test which showed non-significant difference that justifies the accuracy of the models. The desirable formulation of *Aloe vera* fortified apple spread with 60 per cent of apple pulp, 40 per cent of *Aloe vera* pulp (Ap_{60}: Al_{40}), 60.60 ºB of TSS, 0.51 per cent acidity and 0.5 per cent pectin was found best as a result of the responses such as cooking time (775.2 sec), water activity (0.78), TSS (60.60 ºB), spreadability (22.57 cm^2/g/min), appearance (8.50), texture (8.74), flavour (8.75) and overall acceptability (8.73). Whereas, comparatively lower sensory acceptability scores were recorded in the formulations which contained higher amounts of acid and pectin.

5.3.3 The optimized *Aloe vera* fortified apple spread contained water activity of 0.78, 27.39 per cent of moisture content, 24.19 per cent of reducing sugars, 58.66 per cent of total sugars and 0.34 of non-enzymatic browning (NEB). Comparatively higher values of total phenols (77.27 mg/100g), antioxidant potential (55.46 %), potassium (0.163 g/100g), phosphorus (0.489 g/100g), copper (0.67 mg/100g), manganese (1.56 mg/100g) and magnesium (3.05 mg/100g) were recorded in the *Aloe vera* fortified apple spread than that of apple spread.

5.3.4 For the development of *Aloe vera* fortified low calorie apple spread, different proportions of low calorie sweeteners were tried to replace the sucrose with sweetos (fructooligosaccharide + sucralose). It was observed that, the addition of sweetos up to 100 per cent revealed similar sensory scores to that of 100 per cent of sucrose containing spread. The 100 per cent incorporation of sweetos with 0.5 per cent of pectin was found optimum without any adverse effect on overall sensory acceptability of developed spread. Hence, the present study indicated good potential of sweetos for its use in development of low calorie products like spread.

5.3.5 Spread prepared by using 100 per cent replacement of sucrose with equivalent sweetness of sweetos with 0.5 per cent pectin had 51.48 ºB TSS, 0.60 per cent acidity, 29.10 per cent reducing sugar, 42.07 per cent total sugar, 0.26 non-enzymatic browning, 1.25 per cent fat, 1.08 per cent protein, 0.162 g/100g of potassium, 0.478 g/100g of phosphorus and 74.66 mg/100g total phenols.

5.3.6 The low calorie apple spread showed changes in their physico-chemical and sensory quality characteristics when stored in polypropylene cups for a period of six months. Whereas, the changes were negligible in the spreads which were packed in glass jars. The developed products experienced slight increase in moisture content, titratable acidity, pH and microbial load up to six months of storage. Whereas, significant decrease in TSS, total sugars, reducing sugars, ascorbic acid, antioxidant potential and total phenols was recorded after six months of storage in the spread packed in the polypropylene cups. Although, the sensory scores of spreads packed in glass jar were found to decrease during storage, yet were well within the acceptable limits (≥ 7.00). While, in case of the spreads packed in polypropylene cups, the sensory scores decreased drastically after three months of storage and the products were '*disliked*' by the judges.

5.3.7 The calorie value of apple spread, *Aloe vera* fortified apple spread and low calorie apple spread observed as 306.6, 291.63 and 100.27 Kcal/100g, respectively. Spread with 100 per cent sucrose contained approximately double calories as compared to low calorie apple spread. Thus, the low calorie apple spread exhibited about 65 per cent reduction in the energy value over 100 per cent sucrose sweetened spread.

5.3.8 The microbial examination (as total plate count) of the spreads revealed that there was very less bacterial and fungal growth observed in spread packed in glass jars during six months of storage but, the microbial load was quite high and exceeded FSSAI limits in the spread packed in polypropylene cups after six months. Thereby, indicating superiority of glass jars over polypropylene cups for packing and storage of apple spreads up to a period of six months. However, it is recommended that the product can be kept for longer periods under refrigerated conditions.

5.3.9 There was a non-significant difference in the total cost of production of different apple spreads. However, slight differences were recorded in apple spread, *Aloe vera* fortified apple spread and low calorie apple spread packed in the glass jar and polypropylene cups. The cost of apple spread, *Aloe vera* fortified apple spread and low calorie apple spread was calculated as Rs 25.43, Rs 27.51 and Rs 48.81 per glass jar (200g), and Rs 16.63, Rs 18.71 and Rs 40.01 per polypropylene cup (200g).

Conclusively, the findings of present investigation demonstrates the effective way of providing health benefits of both apple and *Aloe vera* with or without replacement of sucrose in the form of fruit spread. The results also suggested that *Aloe vera* fortified apple spread consisting of 60 per cent apple pulp and 40 per cent *Aloe vera* pulp with 60 ºB TSS, 0.5 per cent acidity and 0.5 per cent pectin was optimized as acceptable and healthful product. Further, replacement of sucrose with 100 per cent equivalent sweetness level of sweetos along with 0.5 per cent pectin was found optimum for preparation of palatable low calorie *Aloe vera* fortified apple spread with respect to overall sensory quality. Hence, it can be concluded that *Aloe vera* and sweetos can successfully be utilized for the development of innovative low calorie *Aloe vera* fortified functional apple spread for the benefit of diabetic, obese and health conscious people.

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APPENDIX – I

DUO-TRIO TEST

EVALUATION FOR RELATIVE SWEETNESS OF NON-NUTRITIVE SWEETENER (SWEETOS)

NAME: ______________________ Date: ________________

PRODUCT: ______________________

The first sample 'R' given is the reference sample.

Taste it carefully.

From the pairs of coded samples next given, judge which sample is the same as 'R' A positive answer is to be made even if it is a guess.

Please evaluate the following samples as per the standard scale.

Set No.	**Code No. of pairs**	**Same as 'R'**
I.	**…………………..**	**…………….**
II.	**…………………..**	**…………….**
III.	**…………………..**	**…………….**
IV.	**…………………..**	**…………….**

Signature of Evaluator

APPENDIX – II

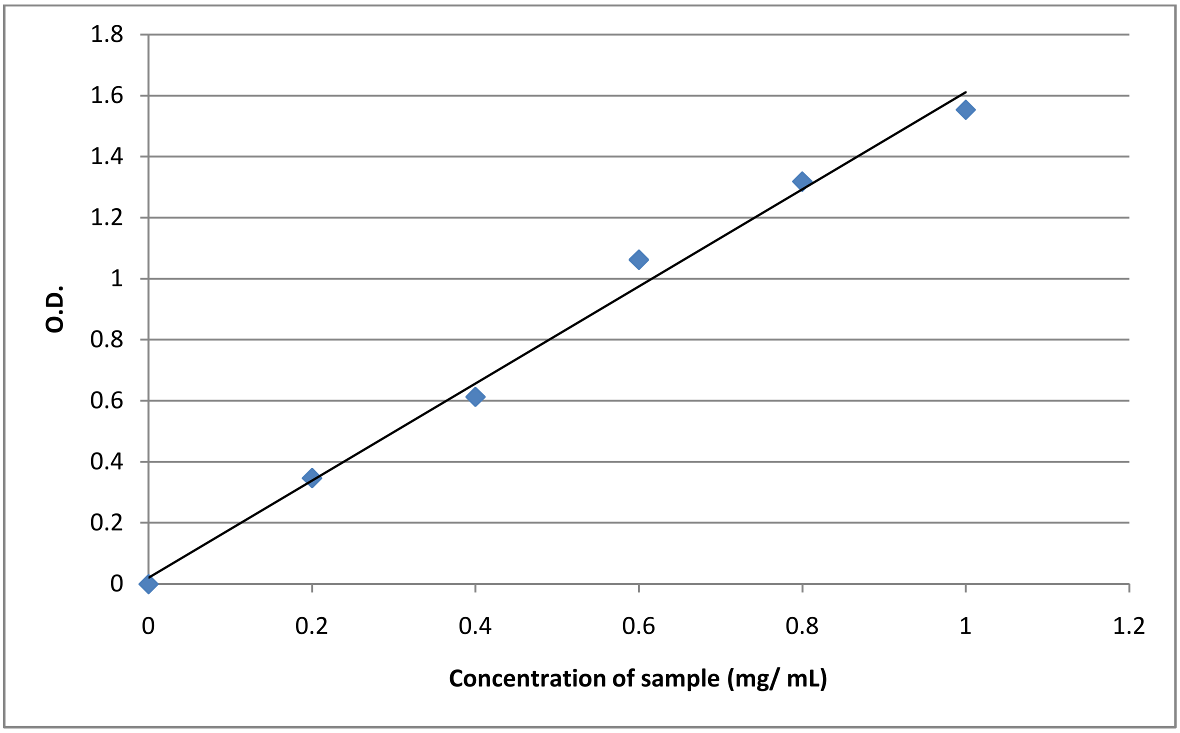
Mean monthly meteorological data recorded during storage study of the products

Month	Temperature (°C)	
	Maximum	Minimum
January, 2020	16.4	3.70
February, 2020	21.3	6.10
March, 2020	22.9	7.80
April, 2020	25.3	13.2
May, 2020	30.5	15.8
June, 2020	34.6	19.2

Source: Meteorological Observatory, Department of Environmental Science, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh

APPENDIX - III

STANDARD CURVE OF GALLIC ACID



APPENDIX – IV

HEDONIC RATING TEST EVALUATION FOR SENSORY QUALITY OF FRUIT SPREADS

NAME: ______________________ Date: ________________

PRODUCT: ______________________

Please evaluate the following samples as per the standard scale.

Attributes / Treatments	Appearance	Texture	Spreadability	Flavour	Overall acceptability

9-point Hedonic scale

9 =Like extremely

8 = Like very much

7 =Like moderately

6 =Like slightly

5 = Neither like nor dislike

4=Dislike slightly

3=Dislike moderately

2=Dislike very much

1=Dislike extremely

Signature of Evaluator

APPENDIX – V

Aloe vera pulp (%)	TSS (ºB)	Acidity (%)	Pectin (%)	Cooking time (sec)	Water activity	TSS (ºB)	Spreadability (cm^2/g/min)	Texture	Appearance	Flavour	Overall acceptability	Desirability	
40	60	0.51	0.50	771.2249	0.807712	60.1983	22.78079	8.812875	8.477958	8.732542	8.698875	0.987982	Selected
40	60	0.56	0.51	770.9258	0.808054	60.25698	22.71993	8.751098	8.442012	8.689878	8.652763	0.985482	
40	60	1.00	0.51	773.1332	0.80834	60.22808	22.67414	8.303192	8.206853	8.481519	8.309515	0.983577	
40	60	0.99	0.50	773.3337	0.808244	60.23669	22.6722	8.317028	8.220943	8.489304	8.321914	0.983505	
40	60	1.00	0.51	773.1495	0.808326	60.23281	22.6718	8.308746	8.211412	8.483943	8.314282	0.983488	
40	60	0.64	0.50	770.6924	0.808319	60.31911	22.65351	8.665396	8.400062	8.637705	8.589616	0.982695	
40	60	1.00	0.96	755.798	0.810746	59.69103	22.65026	7.825692	7.612634	8.033734	7.812586	0.982572	
40	60	1.00	0.94	756.4635	0.81083	59.73029	22.64449	7.846953	7.635081	8.054855	7.835465	0.982349	
40	60	1.00	0.58	770.3142	0.809342	60.21171	22.64295	8.240057	8.112469	8.424004	8.245744	0.982287	
40	60	1.00	0.59	769.8607	0.809479	60.2015	22.64199	8.225251	8.094197	8.412809	8.231342	0.982245	
40	60	1.00	0.93	756.904	0.810881	59.75806	22.63957	7.861942	7.65109	8.069643	7.851576	0.982076	
40	60	0.69	0.50	770.7275	0.808453	60.34096	22.62708	8.614327	8.3734	8.608868	8.551301	0.981583	
40	60	0.91	0.50	772.2973	0.808444	60.30457	22.62521	8.391998	8.26014	8.511445	8.381135	0.98152	
40	60	1.00	0.82	760.9137	0.811013	59.95583	22.61813	7.984031	7.787343	8.188417	7.981547	0.98123	
40	60	1.00	0.72	764.8026	0.810642	60.09524	22.61635	8.094167	7.920515	8.292823	8.096901	0.981178	
40	60	1.00	0.74	764.2546	0.810723	60.07862	22.61551	8.079095	7.901684	8.278757	8.081242	0.981144	
40	60	1.00	0.79	762.2953	0.810943	60.01002	22.61675	8.02481	7.835393	8.227304	8.024477	0.981006	
39.99	60	1.00	0.76	763.3438	0.810821	60.05415	22.61135	8.056302	7.873655	8.257125	8.057503	0.980744	
40	60	0.96	0.67	766.9476	0.810363	60.2068	22.58502	8.186234	8.016088	8.353055	8.185734	0.97987	
40	60	0.81	0.53	770.6526	0.808972	60.35678	22.58235	8.464638	8.27531	8.525761	8.433215	0.979758	
40	60	0.95	0.92	758.6201	0.811051	59.88386	22.57151	7.91159	7.698053	8.087304	7.901866	0.979303	
40	60	0.84	0.58	769.359	0.809713	60.34674	22.55276	8.376643	8.185155	8.462341	8.355223	0.978517	
40	60	0.90	1.00	757.5587	0.810693	59.79453	22.54316	7.839907	7.625518	7.991954	7.825546	0.978054	
40	60	0.69	0.62	769.2335	0.809966	60.37148	22.53761	8.473532	8.217049	8.489586	8.426193	0.977881	
40	60	0.50	0.90	773.4183	0.809917	60.17947	22.50307	8.255224	7.990601	8.252536	8.215032	0.976428	
40	60	0.67	0.72	768.3911	0.810656	60.3468	22.48801	8.364879	8.101641	8.390103	8.325897	0.975792	
40	60	0.53	1.00	772.7023	0.80954	60.06791	22.46139	8.082168	7.858585	8.099086	8.057209	0.974668	
40	59.81	0.88	0.50	769.6146	0.80911	60.15248	22.72278	8.410117	8.258852	8.50535	8.39155	0.972973	
40	59.85	0.50	0.94	772.7449	0.810209	60.00257	22.57449	8.184923	7.933275	8.185802	8.149654	0.969737	
35.75	58.5	1.00	1.00	707.1105	0.813894	58.55656	22.3857	7.52398	7.2789	7.67005	7.49904	0.79668	

APPENDIX – VI

ANOVA for sensory attributes of *Aloe vera* fortified low calorie functional apple spread

Source of Variation	Degree of Freedom	Mean Sum of Square (MSS)				
		Appearance	Texture	Spreadability	Flavour	Overall acceptability
Treatments	12	0.086	0.438	0.285	0.044	0.464
Error	24	0.002	0.075	0.131	0.001	0.014

ANOVA for physico-chemical attributes of apple spread, *Aloe vera* fortified apple spread and *Aloe vera* fortified low calorie function apple spread

Source of Variation	Degree of Freedom	Mean Sum of Square (MSS)						
		Moisture content	TSS	Reducing sugars	Total sugars	Titratable acidity	pH	Ascorbic acid
Packaging material (P)	1	199.65	198.13	82.13	57.54	0.30	0.38	9.055
Storage Intervals (I)	2	18.93	1.08	2.38	3.30	0.41	0.68	162.628
Treatments (T)	2	145.82	453.28	191.42	1386.98	0.05	0.26	3.487
P X I	2	77.03	72.82	30.09	25.38	0.25	0.20	16.393
P X T	2	0.27	0.06	0.09	0.03	0.01	0.02	1.326
I X T	4	0.16	0.79	0.17	0.27	0.01	0.02	1.169
P X I X T	4	0.30	0.10	0.22	0.26	0.01	0.01	1.691
Error	36	29.78	7.76	35.65	32.75	0.03	0.01	2.417

ANOVA for physico-chemical attributes of apple spread, *Aloe vera* fortified apple spread and *Aloe vera* fortified low calorie function apple spread

Source of Variation	Degree of Freedom	Mean Sum of Square (MSS)						
		Total phenols	Anti-oxidants potential	Water activity	NEB	Fat	Protein	Ash content
Packaging material (P)	1	94.92	58.47	0.06	0.01	0.53	0.27	0.58
Storage Intervals (I)	2	1,262.3	817.59	0.00	0.20	1.34	1.85	0.71
Treatments (T)	2	8,344.6	3,655.29	0.02	0.20	3.24	2.25	1.93
P X I	2	25.18	20.38	0.01	0.00	0.13	0.08	0.16
P X T	2	0.95	0.96	0.00	0.00	0.14	0.13	0.06
I X T	4	28.88	4.51	0.00	0.00	0.04	0.15	0.01
P X I X T	4	2.09	0.77	0.00	0.01	0.07	0.08	0.02
Error	36	23.68	12.98	0.01	0.02	0.32	0.29	0.55

ANOVA for sensory attributes of apple spread, *Aloe vera* fortified apple spread and *Aloe vera* fortified low calorie function apple spread

Source of Variation	Degree of Freedom	Mean Sum of Square				
		Appearance	Texture	Spreadability	Flavour	Overall acceptability
Packaging material (P)	1	13.32	4.63	1.94	18.941	12.10
Storage Intervals (I)	2	14.49	6.83	3.26	27.122	15.65
Treatments (T)	2	4.31	12.96	16.10	0.867	4.73
P X I	2	8.84	2.66	1.32	12.895	10.59
P X T	2	0.07	0.13	0.03	0.157	0.01
I X T	4	0.05	0.22	0.01	0.446	0.13
P X I X T	4	0.07	0.13	0.02	0.055	0.03
Error	34	0.60	0.32	0.46	0.727	0.22

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Title of the Thesis	**:**	**Studies on development of *Aloe vera* fortified low calorie functional apple spread**
Name of the Student	**:**	Tamanna Sharma
Admission Number	**:**	H-2018-35-M
Major Advisor	**:**	Dr Rakesh Sharma
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ABSTRACT

In the present study, suitability of *Aloe ver*a and non-nutritive sweetener (sweetos) was evaluated for the development of novel *Aloe vera* fortified low calorie apple spread. First of all, optimization of process for the production of *Aloe vera* fortified apple spread was carried out by using Response Surface Methodology (RSM). The experimental variables considered were *Aloe vera* pulp (10-50%), TSS (45-65^{o}B), acidity (0.25-1.25%) and pectin (0.25-1.25%). As per the design, out of different combinations tried, the *Aloe vera* fortified apple spread prepared by using 40 per cent *Aloe vera* pulp with 60ºB TSS, 0.5 per cent acidity and 0.5 per cent pectin was optimized on the basis of responses recorded in the RSM with 0.988 desirability. The optimized product was further used for the preparation of low calorie apple spread by replacing sugar (sucrose) sweetness with equi-sweetness of sweetos. On the basis of physico-chemical and sensory attributes, the spread prepared by using 100 per cent sweetos with 0.5 per cent pectin level was found most appropriate. The spread so prepared was analyzed for various physico-chemical and sensory characteristics on the preparation day as well as during storage for six months. Results revealed that *Aloe vera* fortified apple spread contained higher amount of total phenols (77.27 mg/100g), potassium (0.163 g/100g), phosphorus (0.489 g/100g) and showed higher antioxidant potential (55.46%) compared to apple spread. Furthermore, the optimized *Aloe vera* fortified low calorie apple spread containing 100.27 Kcal/100g energy value provided about 65 per cent reduction in calories over 100 per cent sucrose sweetened spread. During storage, the spreads packed in glass jars, though experienced marginal changes in their physico-chemical, sensory and microbial attributes, yet remained shelf stable up to six months at ambient temperature. Whereas, maximum changes were obtained in the spreads packed in polypropylene cups and were safe for consumption only up to 3 months. The cost of production of the spreads ranged from Rs. 16.63 to Rs 48.81. Overall, the results of present work provided an effective way of delivering health benefits of both apple and *Aloe vera* to the consumers in the form of palatable and functional spreads with reduced calories. Hence, their availability in the market will definitely benefit the diabetic, obese and health conscious people.

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