CHAPTER I

INTRODUCTION

Medicinal plants and herbs have been proved to be of great importance to the health of the individuals and communities since ancient times and are used for various treatments and therapies. In recent years, many scientific investigations of traditional herbal remedies for several diseases have been carried out and this lead in the development of alternative drug and therapeutic strategies. The use of these plants as a supplement in food taking in to account that these plants can present a significant amount of useful components.

Carica papaya is one of the valuable plants used for various purposes in medicinal field. Leaves, fruit and seeds of the *C.papaya* are used as ethnomedicine. This work describes about **"Development and quality evaluation of *C. papaya* leaves enriched food products"**. Several studies had indicated that the leaf extract of *C.papaya* has high potentiality for curing number of diseases.

Phytochemical screening revealed the presence of bioactive compound saponins, cardiac glycoside, alkaloids and absence of tannins in the in the papaya leaf.

Papaya [Carica papaya Linn. (C. papaya)] is one of the most cultivated plants in tropical countries and the most popular and economically important species among the Caricaceae family . Papaya grows best in a well drained, well aerated and rich organic matter soil, pH 5.5 – 6.7 , Although only the fruits are generally used as commercial produces, in several Asian Pacific countries, the leaves are also used as traditional medicines for treatment of asthma, colic, fever, beriberi (India), malaria and dengue fever (Sri Lanka, Pakistan and Malaysia), and cancer (Vietnam and Australia) . In Indonesia, the leaves are consumed as a vegetable, tea, and traditional medicine (called jamu) for many purposes such as increasing the appetite and breast milk production, reducing fever, and also for preventing and curing malaria.

Fig 1.01 *Carica papaya*

Whole C. papaya i.e. its fruits, seeds, bark and leaves are used for treatment and curing of many disease. The edible portion of the fruit of C. papaya (pawpaw) contains both macro and micro minerals like Na, K, Ca, Mg, Fe, Cu, Zn and Mn. The plant is a source of carotenoids, vitamin C, thiamine, riboflavin, niacin, vitamin B6 and vitamin K . The seed had recently been linked to cure sickle cell diseases , poisoning related renal disorder and as an anti-helminths. The tender leaf has been consumed as an alternative to traditional leafy vegetables and as an additive to tenderise meat.

Papaya leaf has a numberless of benefits. Green papaya leaf is a source of essential nutrients while yellow papaya is a source of iron. Fresh, green papaya leaf is an antiseptic, whilst the brown, dried papaya leaf is the best as a tonic and blood purifier. The leaves of papaya have been shown to contain many active components that can increase the total antioxidant power in blood and reduce lipid peroxidation level, such as papain, chymopapain, cystatin, tocopherol, ascorbic acid, flavonoids, cyanogenic glucosides and glucosinolates .The extracts of both the leaves and fruit are known to contain several proteins and alkaloids with important pharmaceutical, medical, and industrial applications. Papaya fruit juice and leaf extract have demonstrated anti-cancer properties.

Most important traditional use of leaf juice is its capability to increase white blood cells & platelets, normalizes clotting and also repairs the liver. Ayurvedic literature reveals that papaya leaf extract has haemostatic properties and recent studies on ability of C. papaya leaf aqueous extract on platelet augmentation in cyclophosphamide induced thrombocytopenia rat model was studied and found significant effects. Studies have found that the powder from

papaya leaves has substances responsible for the release and production of thrombocytes/platelets and have shown effective in treating dengue viral infections. Papaya leaves can be used as an antihypertensive agent. Leaves contain large amounts of alkaloids, carpaine and pseudocarpine which creates positive effects on heart as well as on respiration. The extracts of papaya leaves accelerate the increase in platelet counts and shorten the hospitalization period during dengue fever. Till now there is no approved vaccine or drug against this dengue virus. Therefore, pawpaw leaves can be manipulated in the herbal treatment of various diseases and as a potential source of useful elements for drugs Papaya leaves can be used as complementary drug in dengue fever. Young leaves of papaya have antioxidant properties and have a depressing action on heart.

Papaya leaf can be used for the processing of green tea. Aqueous extract of papaya leaf tea plays an important role as a tumour destroying agent. Papaya leaf tea is the most powerful anti-cancer agent. Studies have found that the enzymes found in papaya leaf tea have dramatic cancer fighting properties against a broad range of tumours. A recent study by Purdue University showed that Papaya Leaf Tea consists of over 50 active ingredients found to kill fungi, worms, parasites, bacteria, and many forms of cancer cells.

In addition to cancer fighting substances, papaya leaves boast large doses of important nutrients that support the immune system, including vitamins A, C, and E. Most importantly, it contains vitamin B-17, which in concentrated form is already used as part of traditional chemotherapy treatments. Papain, an important enzyme in papaya leaves is also a powerful digestive aid. It breaks down proteins naturally and eases the burden of digestion on the pancreas and stomach. Scientific research shows that papain is most active at higher tea temperatures.

Microencapsulation is a process by which solids, liquids or even gases may be enclosed in thin coatings or wall material around the substances. It provides the means of converting liquids to solids, of altering colloidal and surface properties, of providing environmental protection and creation of a barrier to avoid chemical reactions and/or to enable the controlled release of the coated materials The microencapsulation of active component may be carried out by different techniques such as spray-drying, spray freeze drying, spray-cooling, spray-chilling, freeze-drying, centrifugal extrusion, air suspension coating, extrusion, coacervation, co-crystallization, rotational suspension separation, liposome entrapment, interfacial polymerization, molecular inclusion, etc. Microencapsulation with suitable wall material can

protect the flavour from undesirable interactions with food, reduces off-flavour, minimize the oxidation, increase shelf-life, allow a controlled release and retain aroma in a food product during storage. Among the various methods, spray drying is the most common and economical method to carry out microencapsulation process since the process is simple, relatively inexpensive, rapid, continuous and produces particles of good quality. This method is especially suited for large volume low cost requirements such as in food industry.

Microencapsulation by spray drying is a fast and continuous dehydration process in which the feed solution is transformed into a solid powder forms a continuous matrix surrounding the active substances of micro particles after short drying period. The feed liquid (solution, emulsion or suspension) containing wall material and core material are sprayed into heated air in the spray drier. The solvent from the feed is evaporated to give instantaneous powder. The heated air supplies the latent heat of vaporization required to remove the solvent from the wall material, thus forming the microencapsulated product.

The work involves determining the qualitative and quantitative analysis of various compounds present in *C. Papaya* leaves of different maturity stages (tender, intermediate, mature) through different extraction procedure (Hot extraction, cold extraction, fresh juice) for each maturity stage. The objective involved the selection of best combination of maturity stage and extraction method to obtain the best results of active components in the leaf. Then the use of spray drying technology to microencapsulate the powder. Again the qualitative and quantitative analysis of the components were carried out over the spray dried powder to confirm its presence and evaluate the presence of those components from extract to the powder along with the development of various *C.Papaya* leaves enriched products.

Chapter 2

REVIEW OF LITERATURE

This chapter sets out to identify and critically analyse all the previously published literature with regard to the general information of papaya, extraction and analysis of active components in papaya leaf of different maturity indices, micro-encapsulation of active components by spray drying and product development from encapsulated powder.

2.1 Papaya leaf (Carica papaya L.)

The papaya (*Carica papaya* L.) is a tropical fruit that is widely cultivated and consumed, both for its agreeable flavour as well as its many pharmacological properties. (J.G de Oliveira, 2011).

Papaya is grown in nearly all countries of the tropical Americas (Central and South America and the state of Hawaii). It is also cultivated in India, Sri Lanka, various Asian countries, as well as the Antilles and tropical Africa (Chan & Paull, 2008).

2.1.1 Production

The papaya cultivated in area of 73700 ha, of which Kerala has the largest area of cultivation of about 13200 ha and production around 59.7 million tonnes.

State	**Area ('000 Ha.)**	**Production ('000 MT)**	**Productivity (MT/Ha.)**
Andhra Pradesh	11.7	1173.6	100.0
West Bengal	7.2	241.9	33.5
Karnataka	3.6	238.1	65.5
Orissa	10.7	217.5	20.3
Gujarat	4.4	175.1	39.4
Maharashtra	5.8	174.4	30.0
Assam	7.5	111.8	14.8
Kerala	13.2	59.7	4.5
Madhya Pradesh	0.8	39.2	49.0
Others	8.6	159.1	-
TOTAL	**73.7**	**2590.4**	**35.1**

Table 2.01: State-wise Area, Production & Productivity of papaya during 2001-02

Source: Database of National Horticulture Board, Ministry of Agriculture, Govt. of India.

Although only the fruits are generally used as commercial produces, in several Asian Pacific countries, the leaves are also used as traditional medicines for treatment of asthma, colic, fever, beriberi (India), malaria and dengue fever (Sri Lanka, Pakistan and Malaysia), and cancer (Vietnam and Australia) (Nguyen & Parat, 2016)

2.1.2 Medicinal values

India has rich medical heritage with a large number of traditional practices, systems and medicines as a part of its total health care scenario, some of them are more than 3,000 years old. In spite of remarkable achievements of modern medicines and research, these ancient systems continue to play a major role in the control or alleviation of diseases. In India several scientists have reported the therapeutic importance of the chemical constituents of plants used in ancient Indian medical system. Mutalic G. (1972) has emphasized for research in traditional medicine.

In spite of the concurrent use of the extract of Carica papaya with prescription oral hypoglycemic agents in some patients there is a dearth of literature on the effects of the extract on activity of oral hypoglycaemic agents (Fakeye, T.O. et al., 2007 and Oyelola O., 2005).

It has been reported by Parle Milind et.al. (2011) that whole papaya plant is useful in various medicinal properties like uterotonic, nephroprotective, anti-inflammatory, anti-tumour, etc.

There is several medicinal importance of the whole plant. Papaya also has several industrial uses. Biochemically, its leaves and fruit are complex, producing several proteins and alkaloids with important pharmaceutical and industrial applications Parle Milind et.al. (2011).

In Indonesia, the leaves are consumed as a vegetable, tea, and traditional medicine (called jamu) for many purposes such as increasing the appetite and breast milk production, reducing fever, and also for preventing and curing malaria. (Julianti & Oufir, 2014).

Many in-vitro and in-vivo studies have demonstrated the medicinal properties of the extracts of papaya leaves including anti-dengue (Ahmed & Fasal, 2011) , anti-plasmodial (

Julianti & de Meiri, 2014), anti-cancer (Liew & Stanbridge, 2012) antibacterial (Bhaskaran & Radha Bai, 2012), hepatoprotection [9], anti-inflammatory [10] and antioxidant [11].

2.1.3 Phytochemical components of Papaya leaf

Phytochemicals and their derived products have been an extraordinary source of compounds with therapeutic and drug development potential (De C et al., 2012).

These molecules are novel and complex structures that can be used in their original form, or can serve as lead molecules to develop derivatives with higher specificity and fewer side effects (Koehn & Carter, 2005).

The World Health Organization has been particularly attentive to the potential offered by herbal medicine, the main subfield of traditional medicine practiced in different countries (WHO, 2012).

Leaves contain large amounts of alkaloids, carpaine and pseudocarpine which creates positive effects on heart as well as on respiration (Perry and Metzger, 1980). Leaf extract of C. papaya is well known as an anti-tumour agent (Walter Last, 2008).

Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids (Edogwa & Okwu, 2005). These compounds are synthesized by primary or rather secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas (Vasu & Goud, 2009).

A large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effects on all types of microorganisms in vitro (Cowan M.M, 1999).

Plant products have been part of phytomedicines since time immemorial. This can be derived from barks, leaves, flowers, roots, fruits, seeds. (Criagg & David, 2001). Knowledge of the chemical constituents of plants is desirable because such information will be value for synthesis of complex chemical substances (Parekh & Chanda, 2008).

The leaves of papaya have been shown to contain many active components that can increase the total antioxidant power in blood and reduce lipid peroxidation level, such as

papain, chymopapain, cystatin, tocopherol, ascorbic acid, flavonoids, cyanogenic glucosides and glucosinolates (Noriko Otsuki et. Al., 2010)

Reports on different parts of C. papaya have been published, but still a comparative study is to be needed to study the difference in quantity of active components in leaves of different maturity indices.

2.2. Extraction of active components in papaya leaf

Purification and isolation of bioactive compounds from plants is a technique that has undergone new development in recent years (Altemimi & Watson, 2015).

The extraction procedures are vital important in analysis of phytochemicals. There are some traditional extraction methods and novel extraction methods. Maceration, percolation and Soxhlet extraction methods are prominently used in phytochemical screening studies. But there are some advanced methods such as supercritical fluid extraction (SFE), microwave assisted (MAE), ultrasound-assisted extraction (UAE) and accelerated solvent extraction. (Azwanida N.N., 2015).

Phytochemicals can be extracted more when the leaves are dried and pulverized to powered form. (Ikeyi Adachukwu P et. al., 2013)

The impact of different types of solvents, such as methanol, hexane, and ethyl alcohol, for the purpose of antioxidant extraction from various plants parts, such as leaves and seeds. In order to extract different phenolic compounds from plants with a high degree of accuracy, various solvents of differing polarities must be used (Wong & Kitts, 2006)

Extraction from the plant is an empirical exercise since different solvents are utilized at varying conditions such as time and temperature of extraction. Cold extraction can be done by drying different parts of plants in an artificial environment at low temperature (50-60 C) and dried powder then further used for extraction purpose using various solvents. (Chapman & Hall, 1973).

Universal Extraction System (Buchi) is recently used for solvent extraction. The dried powder of various plant parts placed in glass thimble for extraction purpose using various solvents. The procedure is carried out for 10 cycles for each extract and adjusts the temperature just below the boiling point of the respective solvents. The resulting solvent extract is filtered,

concentrated in vacuum concentrator and used to determine the presence of phytoconstituents. (Patil & Shettigar, 2010)

Different solvents can be used depending on the kind of phytochemicals that are targeted for extraction. Solvents differ in polarity, just like phytochemicals. There are three polarity strengths of solvents and they are polar, medium polar and non-polar. Polar solvents will extract polar compounds and the same is true for non-polar solvents. Polar solvents include methanol, ethanol and water. Thus, in a sample, different solvents can be mixed for extraction or they can be used in sequence in the same sample material. (Lorato Lekgari, 2010)

2.2.1 Qualitative analysis

Chemical tests are performed on different extracts of plants with standard methods for various secondary metabolites. (Gupta et.al., 2013)

Standard protocols were used for qualitative analysis of alkaloids, flavonoids, saponins, tannins, phenols, proteins, cardiac glycerides, terpenoids carbohydrates in Cissus quadrangularis. (Jayaramu M. et. al., 2016).

The Phytochemical analysis of the leaves showed that the leaves contained saponins, cardiac glycosides, and alkaloids. Tannin was absent in the leaves (Okwu & Okwu, 2004).

Alkaloids, flavonoids, saponins, tannins, steroids and/or terpenes (triterpenoids), and quinones were identified. (Isela & Carlos, 2014).

Carica papaya leaves contain Alkaloid, Saponin, Tannin, Glycoside and Flavonoids [Willson et al., 2007].

2.2.2 Quantitative analysis

Analytical method for quantitative determination of phytochemical components like alkaloid, phenol etc., were according to Jayaramu M. et al., (2016).

Brun *et al.* (1993) concluded that the quantity of chemical substances varies in the fruit, latex, leaves, and roots and varies with the extraction method, age of the plant part, and the cultivar and sex of the tree.

2.3 Microencapsulation

According to Rosenberg et al. (1985), microencapsulation is a processing method in which small quantities of solid, liquid and gaseous materials are packed into a wall matrix; which forms microcapsules.

Shahidi and Han (1993) proposed six reasons for applying microencapsulation in food industry to reduce the core reactivity with environmental factors; to decrease the transfer rate of the core material to the outside environment; to promote easier handling; to control the release of the core material; to mask the core taste; and finally, to dilute the core material when it should be used in only very small amounts.

Lin et al. (1995) conducted a study on microencapsulation of squid oil with hydrophilic macromolecules for oxidative and thermal stabilization. Gelatin, sodium caseinate, and maltodextrin were the wall materials used. The oxidative and thermal stabilities of crude squid oil have been effectively enhanced by spray drying microencapsulation.

According to Sheu and Rosenberg (1995), microencapsulation was a process where droplets core were coated by thin films which protect the core until it needed. During microencapsulation the entrapment of sensitive ingredients within a continuous film or coating could protect them from environmental factors such as moisture, air or light (Onwulata et al., 1995).

Jain et al. (1997) stated that microencapsulation is a technology of packaging solids, liquids or gaseous material in miniature sealed capsules that could release their contents at controlled rates at specific conditions. The miniature packs in packages, called 'microcapsules', may range from micron to several millimetres in size and was ideally spherical.

Gibbs et al. (1999) narrated three precautions for developing microcapsules: formation of the wall around the material, ensuring that leakage does not occur and ensuring that undesired materials are kept out for which encapsulation techniques such as spray drying, spray chilling or spray cooling, extrusion coating, fluidized-bed coating, liposomal entrapment, lyophilization, coacervation, centrifugal suspension separation, co-crystallization and inclusion complexation could be used.

Microencapsulation is the technique by which one material or a mixture of materials is coated with or entrapped within another material or system. The coated material is called active or core material, and the coating material is called shell, wall material, carrier or encapsulant.

The simplest of the microcapsules may consist of a core surrounded by a wall or barrier of uniform or non-uniform thickness. The core may be composed of just one or several types of ingredients and the wall may be single or double-layered (Augustin et al., 2001).

According to Desai and Park (2005) microencapsulation process is used in food industry for several reasons: 1) encapsulation (entrapment) to protect the core material from degradation by reducing its reactivity to its outside environment (e.g., heat, moisture, air, and light); 2) to decrease/retard the evaporation or transfer rate of the core material to the outside environment; 3) to avoid alterations in the physical characteristics of the original material, that can be modified easier to handle; 4) the product can be tailored to either release slowly over time or at a certain point (i.e., to control the release of the core material to achieve the property delay until the right stimulus); 5) to mask the flavour of some substances that are microencapsulated and used as the core material; 6) the core material can be diluted when only very small amounts are required, yet still achieve a uniform dispersion in the host material, and to separate components in a mixture that would otherwise react with one another.

Shaw et al. (2007) conducted a study on spray dried multi-layered emulsions as a delivery method for ω-3 fatty acids into food systems. It was found that microencapsulation of menhaden oil-in-water emulsions stabilized with a multilayer system consisting of lecithin and chitosan could be an effective technology to produce ω-3 fatty acid delivery system for use in functional foods.

Parize et al. (2008) microencapsulated the natural urucum pigment with chitosan by spray drying in different solvents. This study demonstrates that urucum pigment can be successfully incorporated into chitosan by means of a spray-drying process, resulting in dry and colourful powders which are water soluble.

Lezama et al. (2012) microencapsulated non-aqueous extracts from chilli (Capsicum annuum L.) by spray drying. They reported that approximately 80% of the antioxidant activity of the non-aqueous extracts was preserved in microencapsulates. The microcapsules did not contain fractures, and this finding may have contributed to the protective action against oxidation.

Balasubramani et al. (2013) conducted a study on microencapsulation of garlic (Allium sativum L.) oleoresin by spray drying. The study was carried out with variable core material concentrations (10, 20 and 30%), drying inlet air temperatures (180, 200 and 2200C) and different wall material concentrations (40, 50 and 60%). The optimum conditions obtained for

microencapsulation of garlic oleoresin were, core material at 10% concentration, wall material .at 60% concentration and drying inlet air temperature of 220 0C. Hundre et al. (2015) microencapsulated vanillin extract with different wall materials like β-cyclodextrin (β -cyd), whey protein isolates (WPI) and combinations of these wall materials (β -cyd + WPI). It was revealed that microencapsulation of vanillin is an ideal technique to increase its stability and functionality.

2.3.1Maltodextrin

Ananda Raman and Reineccius (1986) encapsulated orange peel oil by spray drying process. They reported that higher DE (dextrose equivalents) maltodextrin form a denser and more oxygen impermeable matrix providing longer shelf life for orange peel oil.

Maltodextrin is made up of partially hydrolyzing maize starch with acids or enzymes and they are supplied at different dextrose equivalents which is a measure of degree of hydrolysis of starch polymer (Kenyon and Anderson, 1988).

Raja et al. (1989) showed that maltodextrin with dextrose equivalence between 10 and 20 fit in for using as wall material. Those maltodextrin samples show the highest retention of flavour because they could be dispersed in water up to 35.5% of the solution without haze formation.

According to McKernan (1992), maltodextrin was being increasingly used in microencapsulation studies as they had acceptable food grade qualities. They were found to be non- reactive with core materials with low viscosity at high solid content and were relatively inexpensive.

Shahidi and Han (1993) reported that maltodextrin improves the shelf life of citrus oils because maltodextrin had low flavour, and can be used at high solid concentration. Blending of corn syrup solids, maltodextrin and modified starch may lead to optimal microencapsulating materials. Spray drying and extrusion process of the individual components have been used as water soluble coating techniques.

Gibbs et al. (1999) reported that the most commonly used materials for microencapsulation are maltodextrin of different dextrose equivalents. Maltodextrin are obtained by acid hydrolysis of several starches (corn, potato or others). Maltodextrin have high solubility in water, low viscosity, bland flavour and colourless solutions and are extensively used in the food industry.

Buffo and Reineccius (2000) optimized gum acacia/modified starches/ maltodextrin blends for the spray drying of flavours. It was revealed that maltodextrin exhibits various favourable characteristics, including high hydro solubility, low emulsifying capacity, low retention of volatiles, and high protection against oxidation.

Usage of maltodextrin as wall material for production of Amaranthus betacyanin pigment significantly reduced the hygroscopicity of the betacyanin extracts enhancing their storage stability (Cai and Corke, 2000).

Belghith et al. (2001) conducted an experiment on stabilization of Penicillium occitanis cellulases by spray drying in presence of maltodextrin. They reported that 1% maltodextrin had a negative effect on enzyme recovery and it completely stabilized cellulases even after a long period (8 months) of storage at 14 30°C. By using maltodextrin, the activity loss during spray-drying is reduced and storage stability improved.

Maltodextrin is a hydrolyzed starch which offers advantages such as low cost, neutral aroma and taste, low viscosity at high solids concentrations and good protection against oxidation (Gharsallaoui et al., 2007).

Maltodextrin had low flavour and were non-reactive with the core component (anthocyanin) used for microencapsulation. It is improved the shelf life of black carrot anthocyanins and maintained the colour and stability during storage (Ersus and Yurdagel, 2007).

Kha et al. (2010) conducted a study on effects of spray drying conditions on the physicochemical and antioxidant properties of the Gac fruit aril powder. Spray drying of this material has not been successful and maltodextrin is considered as a suitable drying aid to preserve its colour and antioxidant properties. Moisture content and bulk density, colour characteristics, total carotenoid content (TCC), encapsulation efficiency and total antioxidant activity (TAA) were significantly affected by maltodextrin concentration.

The microstructural analysis of the microspheres obtained with different carriers, the use of maltodextrin resulted in more homogeneous particles, which is recommended in the spray drying microencapsulation process (Silva et al., 2013).

Fernandes et al. (2014) microencapsulated rosemary essential oil with gum arabic, starch and maltodextrin as wall material. The mixture of modified starch and maltodextrin proved to be an effective matrix for retaining rosemary essential oil. Maltodextrin is used in

combination with another encapsulating agent because it does not have an emulsifying capacity; it has the advantage of being relatively inexpensive and provides excellent protection of the encapsulated materials.

2.3.2 MICROENCAPSULATION BY SPRAY DRYING

The production of microencapsulated powders by spray-drying generally involved the formation of a stable emulsion in which the wall material acted as a stabilizer for the core material. The emulsion was then spray-dried to yield the encapsulated powder product (Reineccius, 1988).

Deis (1997) reported that spray drying was one of the commercial processes which was widely used in large-scale production of encapsulated flavours and pigments. Spray drying provides a very large surface area, which enhances oxidation, if the wall material is not thick or dense enough to act as a good oxygen barrier.

Desobry et al. (1997) compared the freeze drying and spray drying techniques and reported that spray drying is the most common and cheapest technique to produce microencapsulated food materials. Equipment is readily available and production costs are lower than most other methods. Compared to freeze drying, the cost of spray-drying method is 30–50 times cheaper.

Tari and Singhal (2002) reported that microencapsulation by spray drying can protect the flavour from undesirable interactions with food, minimize loss against light-induced reactions and oxidation, increase the flavour shelf life, allow a controlled release and retain aroma in a food product during storage.

The spray drying process involves the dispersion of the substance to be encapsulated in a carrier material, followed by atomization and spraying of the mixture into a hot chamber (Watanabe et al., 2002). The resulting microcapsules were then transported to a cyclone separator for recovery.

Ersus and Yurdagel (2007) studied microencapsulation of black carrot anthocyanin using spray drying and reported that maltodextrin had low flavour and were non-reactive with the core component (anthocyanin) used for microencapsulation. It improved the shelf life of black carrot anthocyanins and maintained the colour and stability during storage.

Obon et al. (2009) found that betacyanin pigment obtained from Opuntia stricta microencapsulated by co-current spray drying of O.stricta fruit juices with bench-scale two fluid nozzle spray dryer showed high colour strength when stored at room temperature for one month. He reported that spray dried food powders show high storage stability, good handling characteristics (for some applications) and minimized transportation weight in comparison with liquid concentrates. Spray drying is a common method of encapsulation of food ingredients in the food industry.

Spray drying produces, depending on the starting feed material and operating conditions, a very fine powder (10-50 μm) or large size particles (2-3 mm), which are separated in a cyclone after their formation (deVos et al., 2010).

2.4. Development of products

Carica papaya leaves aqueous extract exhibited potential activity against Dengue fever. Furthermore, the different parts of this valuable specie can be further used as a strong natural candidate against viral diseases. (Nisar Ahmad et al., 2011)

Dried and pulverized leaves are sold for making tea, also the leaf decoction is administered as a purgative for horses and used for the treatment of genito-urinary system. (Adebiyi et al., 2002).

The future appears to be bright for the mankind regarding dengue epidemic control, considering the positive results of papaya leaf use, its easy availability and affordability. (Ghan Shyam et al., 2016)

Chapter 3

MATERIALS AND METHODS

Encapsulation procedure of *Carica papaya* leaves and development of products are described in detail.

3.1 Raw material

Carica papaya leaves of different maturity stages were collected from KCAET campus premises for the qualitative and quantitative evaluation of active components such as flavonoids, phenols, alkaloids, etc. and for determining other properties such as antioxidant activity, microbial activity, etc. The analysis were conducted on both on leaf extract obtained by different methods (hot extraction, cold extraction, fresh juice) and also on spray dried leaf powder.

The collected Leaves were graded into tender leaf, intermediate leaf and mature leaf based on parameters such as such as size, length, width, colour etc.

Mature leaves	Less than 25cm
Intermediate leaves	32±4 cm
Tender leaves	More than 37cm

Table 3.01: Maturity indices of different samples

Collected leaves were properly examined to obtain healthy ones, they were then properly washed, cleaned and weighed.

3.2 Leaf drying

The most common and fundamental method for post-harvest preservation of medicinal plants because it allows for the quick conservation of the medicinal qualities of plant material in an uncomplicated manner. Conventionally, low drying temperature between 30°C and 55°C are recommended to protect sensitive active ingredients.

3.2.1 Sun drying

The cleaned healthy leaves were initially dried under sun light for 1.5 hours and then weighed.

3.2.2 Tray drying

The sundried leaves were shredded into small pieces and they were loaded for drying in tray drier at 55°C for 12 hours.

3.3 Grinding

The dried parts were ground to coarse powder with the help of blender. This process breaks the plant parts to smaller pieces thus exposing internal tissues and cells to solvents thus facilitating their easy penetration into the cells to extract the constituents. Then the powdered sample was kept in clean closed glass containers till extraction. During grinding of sample, the grinder was thoroughly cleaned to avoid contamination with any remnant of previously ground material or other extraneous matters deposited on the grinder.

3.4 Packaging of powder

The ground powder of different maturity were packed separately in polythene cover, sealed using continuous band sealer and stored at ambient temperature.

The weight of different samples after the above procedures are shown below.

	Fresh weight (g)	After sun drying (g)	After cutting (g)	After tray drying(g)	Powder (g)
Mature leaves	412.3	372.5	359.7	84.5	83
Intermediate leaves	312.1	281.0	278.4	44.6	41.5
Tender leaves	183.2	172.9	167.9	22.9	24.7

Table 3.02: Weight of sample after different drying procedure

3.5 Preparation of solvent extracts

All the extracts were prepared using distilled water as solvent. The adopted extraction procedure includes cold extraction, hot extraction, and fresh juice preparation.

3.5.1 Cold extraction

Cold extraction using water as solvent were carried out by using 10g of each coarsely powdered leaf sample with 100ml of water and kept for 48 hours with slight shaking at162 rpm in rotary shaker incubator at room temperature. After 48 hours, the extracts were filtered into conical flask and the filtrates were kept aside. Again, a 100ml of water was added to the residue and the above procedure was repeated for all the three-maturity leaf powder. Both the filtrates were mixed and final Solute to solvent ratio is 1:20.

3.5.2 Hot extraction

Hot extraction of dried leaves was carried out using 10g of each coarsely powdered sample leaves with 100ml of water and kept for 48 hours in water bath at 55°C. After 48 hours, the extracts were filtered in a conical flask, and the filtrates were kept side. Again, a 150ml of water was added to the residue and the above procedure was repeated for all the three-maturity leaf powders. Both the filtrates were mixed to get a Solute to solvent ratio of 1:25.

3.5.3 Fresh leaf juice

The juice was prepared from fresh leaves. Leaves of different maturity (mature, intermediate and tender) was collected, washed and ground using distilled water as solvent in juice maker. About 25g of each leaf sample was taken and blended with 250ml of distilled water to prepare the fresh juice.

3.6 Phytochemical analysis

Plant kingdom harbours an inexhaustible source of active ingredients invaluable in the management of many intractable diseases. Phytochemical techniques played a significant role in searching raw materials and resources for pharmaceutical industry. Preliminary Phytochemical tests are helpful in finding and locating chemical constituents which are source of pharmacologically active principles. Qualitative phytochemical analysis for studying the presence of active compounds like Alkaloids, Carbohydrates, Phytosterols, Saponins, Glycosides, Phenols, Flavonoids, Diterpenes, Protein & amino acids. Successive isolation of phytocompounds from plant materials depended on the type of solvent used in extraction procedure. The qualitative changes in the phytochemical analysis of tested plant species are correlated to methods of preparation [Pandith, 2012].

Both qualitative and quantitative analysis of the components such as alkaloids, phenols, flavonoids, etc. were conducted specifically on the three extracts.

3.6.1.2 Qualitative analysis

Following standard protocols were used for qualitative analysis of samples to check for the presence of Alkaloids, Carbohydrates, Cardiac glycosides, Flavonoids, Phenols, Saponins, Tannins, Terpenoids, Quinones and Proteins.

3.6.1.2.1. Test for flavonoids

Flavonoid is one of the secondary metabolites which are ubiquitous in photosynthesis cells. The test for confirming the presence of flavonoids in papaya leaves were conducted as: -

2 ml of each extract was added with few drops of 20% sodium hydroxide, formation of intense yellow colour is observed. To this, few drops of 70% dilute hydrochloric acid were added and yellow colour was disappeared. Formation and disappearance of yellow color indicates the presence of flavonoids in the sample extract.

3.6.1.2.2 Test for saponins

Saponin a group of phytochemicals that can be found in variety of plant food, have been shown to have a number of protective effects on human body including lowering cholesterol, warding off cancer, prevent heart disease etc. To confirm its presence were done by: -

To 2 ml of each extract, 6 ml of distilled water were added and shaken vigorously, formation of bubbles or persistent foam indicates the presence of saponins.

3.6.1.2.3. Test for tannins

Tannin is a group of pale yellow to light brown amorphous substance, responsible for the astringency, colour and some of the flavour. To detect it’s presence the following procedure is carried out: -

To 2 ml of each extract, 10% of alcoholic ferric chloride was added; formation of brownish blue or black colour indicates the presence of tannins.

3.6.1.2.4 Test for phenols

Phenols are plant secondary metabolites whose important features include antioxidant etc. Their presence was confirmed by: -

To 2 ml of each extract, 2 ml of 5% aqueous ferric chloride were added; formation of blue color indicates the presence of phenols in the sample extract.

3.6.1.2.5 Test for proteins

To 2 ml of each extract, 1 ml of 40% sodium hydroxide and few drops of 1% copper sulphate were added; formation of violet colour indicates the presence of peptide linkage molecules in the sample extract.

3.6.1.2.6 Test for cardiac glycosides

To 1 ml of each extract, 0.5ml of glacial acetic acid and 3 drops of 1% aqueous ferric chloride solution were added, formation of brown ring at the interface indicates the presence of cardiac glycosides in the sample extract.

3.6.1.2.7 Test for terpenoids

Terpenoids are important for plant survival and also possesses biological and pharmacological properties that are beneficial to human. To test their presence: -

Take 1 ml of extract of each solvent and add 0.5 ml of chloroform followed by a few drops of concentrated sulphuric acid, formation of reddish-brown precipitate indicates the presence of terpenoids in the extract.

3.6.1.2.8 Test for carbohydrates

Take 1 ml of extract, add few drops of Molisch's reagent and then add 1 ml of concentrated sulphuric acid at the side of the tubes. The mixture was then allowed to stand for 2 to 3 minutes. Formation of red or dull violet colour (a ring formation) indicates the presence of carbohydrates in the sample extract.

3.6.1.3 Quantitative analysis

Depending on the above qualitative results the quantitative assay is carried out for Alkaloids, flavonoids, Phenols.

3.6.1.3.1 Total Phenol Content Determination

The phenols were determined by slightly modified Folin and Ciocalteu method. Briefly, to the 200µl of the sample extract, 800 µl of Folin Ciocalteu reagent mixture and 2 ml of 7.5% sodium carbonate added. The total content is diluted to 7 volumes with distilled water and finally kept the tubes for 2 hours incubation in dark. The absorbance was measured at 760 nm. Gallic acid dilutions were used as standard solutions. The results of phenols are expressed in terms of Gallic acid in mg/ml of extract.

3.6.1.3.2 Total Alkaloid Content Determination

A part of extract residue was dissolved in 2N HCL and then filtered. 1 ml of this solution was transferred to separatory funnel and washed with 10 ml chloroform (3 times). The pH of this solution was adjusted to neutral with 0.1 N NaOH. Then 5 ml of BCG solution and 5 ml of phosphate buffer were added to this solution. The mixture was shaken and complex extracted with 1, 2, 3- and 4-ml chloroform by vigorous shaking, the extract was then collected in a 10 ml volumetric flask and diluted with chloroform.

3.6.1.3.3. Total Flavonoid content

Total flavonoid content was measured by the Aluminium chloride colorimetric assay. An aliquot (1ml) of extracts and standard solution of Catechin(100mg/ml) was added to 10ml volumetric flask containing 4ml of distilled water. To this 0.3ml 5% $NaNO_2$ was added. After 5 minutes 0.3 ml of 10% $AlCl_3$ was added. Then after 1 min, 2 ml of 1M NaOH was added and total volume was made up to 10ml with distilled water. The solution was mixed well and absorbance was measured against prepared reagent blank at 510nm. Total flavonoid content of leaf extract expressed as milligram catechin equivalence (CE)/100G fresh weights. All samples were analyzed in triplicates.

3.7. SPRAY DRYING TECHNOLOGY

Spray drying process widely used in food industry for producing dried powdered products could be effectively used for microencapsulation. Spray drying technique for microencapsulation is advantageous in terms of producing free flowing powders with a controlled particle size range at a fast-drying rate at comparatively economical rates enabling scale up of the process for industrial production once the process parameters are standardised.

In this research work, a tall type spray drier with two fluid nozzles having a water evaporation capacity of 1 l/h (M/s S.M. Scientech, Kolkata) was used for the production of microencapsulation of vanilla extract powder. The major components of the spray drier are: air supply system, feed supply system, atomizer, drying chamber, powder recovery system and control panel.

3.7.1 Hot Air Supply System

Air supply system consists of compressor, air filter and air heater. The air is compressed by a compressor and this compressed air is introduced into twin fluid pressure nozzle atomizer after passing through an air filter and heater. The compressed air disintegrates the feed emulsion into a fine mist. An air filter is essential to cease the entry of microorganism. The air is heated through electric heating coils to get a maximum temperature up to 3500℃

3.7.2 Feed Supply System

The feed supply system consists of a peristaltic pump and a feed source. A five hundred millilitre beaker with emulsion was considered as a feed source, the feed which is pumped into the atomizer at the top of the spray drier by a peristaltic pump consisting of five rollers, which squeeze the Hypalon natural rubber tube (6 mm diameter) against the walls of the pump, thereby the feed solution inside the tube was pumped forward in the pumping direction due to the vacuum created sucking the feed solution from the beaker. The motorised peristaltic pump has variable speed arrangement to control the flow. The motor is DC operated and its rpm is controlled by a rotary knob.

3.7.3 Atomizer

The feed solution was introduced into the main chamber in the form of fine spray by means of a two-fluid nozzle from the ceiling of main chamber in downward direction. Compressed air was also introduced into the two-fluid nozzle. The kinetic energy of compressed air is utilized to disperse the feed solution in the form of a fine mist. Two fluid nozzle atomizer has ability to produce a wide range of flow rate and droplet size. It produces a cone shaped spray pattern. The pressure of the compressed air for the flow of the spray was around 2 kg/cm^2. The feed coming from the peristaltic pump was brought into contact with the heated air after atomization for the evaporation of moisture to take place uniformly from the surface of all droplets within the drying chamber

3.7.4 Drying Chamber

The drying chamber of the spray drier is made up of SS304 stainless steel and is cylindrical in shape and the bottom portion is conical for easy flow of dried powder. The atomizer is placed at the top most portion of the drying chamber. Two inspection window glasses are provided at two sides of wall, of which one with 100 W light to see the operation inside the drying chamber. A glass bottle of 40 1000 ml is flanged at the bottom through Teflon gaskets at the conical portion of the drying chamber for collecting the dried powder.

3.7.5 Powder Recovery System

Fine powder particles from the drying chamber were carried along with the hot air and enter into the cyclone separator where they are separated. Air along with particles swirl in a spiral direction down the cyclone and, due to density difference, air leaves the cyclone through a duct pipe since it is less/denser than the particles. The fine powder separated from the cyclone separator is collected in glass bottle attached at the bottom of the cyclone through threaded flange with a Teflon gasket.

3.7.6 Control Panel

The blower speed, feed rate and inlet and outlet temperature were controlled through an electrical control panel with appropriate regulators, ON/OFF push buttons and indicators. In addition to this an automatic and manual de-blocking knob is also connected to solve the clogging of atomizer.

3.8. Spray drying of cold intermediate leaf extract

Among the intensive drying technologies which are used to dry materials, spray drying is one of the most widely applied technology used to dry herbal extracts, due to

1. Large surface obtained by spraying provides effective environment for proper drying.

2. Short drying time allows drying of heat sensitive material as well.

3. Assures continuous operating conditions from inlet to outlet.

A laboratory model vertical co-current SMST tall type spray dryer with an evaporation rate of 1000ml per hour was used. It consisted of an air filter, air heater, air distributor, fluid nozzle, drying chamber, collection glass bottles, cyclone separator and an air compressor.

The factors such as inlet air temperature, outlet air temperature, feed rate and atomizer speed influence the quality of dried powder. Initially distilled water was pumped into the drying chamber to adjust the spray drying temperature, prior to feeding. This is followed by standardization of spray dryer parameters.

The optimization of spray drying parameters has an important role in the production of good quality product along with better yield. Parameters such as inlet temperature, outlet temperature, feed pump rpm, main blower rpm etc. are optimized.

The intermediate leaves were collected in bulk and tray dried at 60℃ as mentioned above and the cold extract was prepared. The TSS (Total Soluble Solids) present in the extract was determined and it was found to be 6° (6-degree brix).

The brix of solution was made to 15°-20° by addition of maltodextrin by 15% by weight of the amount of extract present. The solution was mixed uniformly by using a magnetic stirrer for 10 minutes to prepare a homogenized solution. This solution was highly filtered and fed into the spray drier. The feed rate of spray drier is 4 and inlet temperature is maintained at 185℃ with a blower speed of 1523rpm.

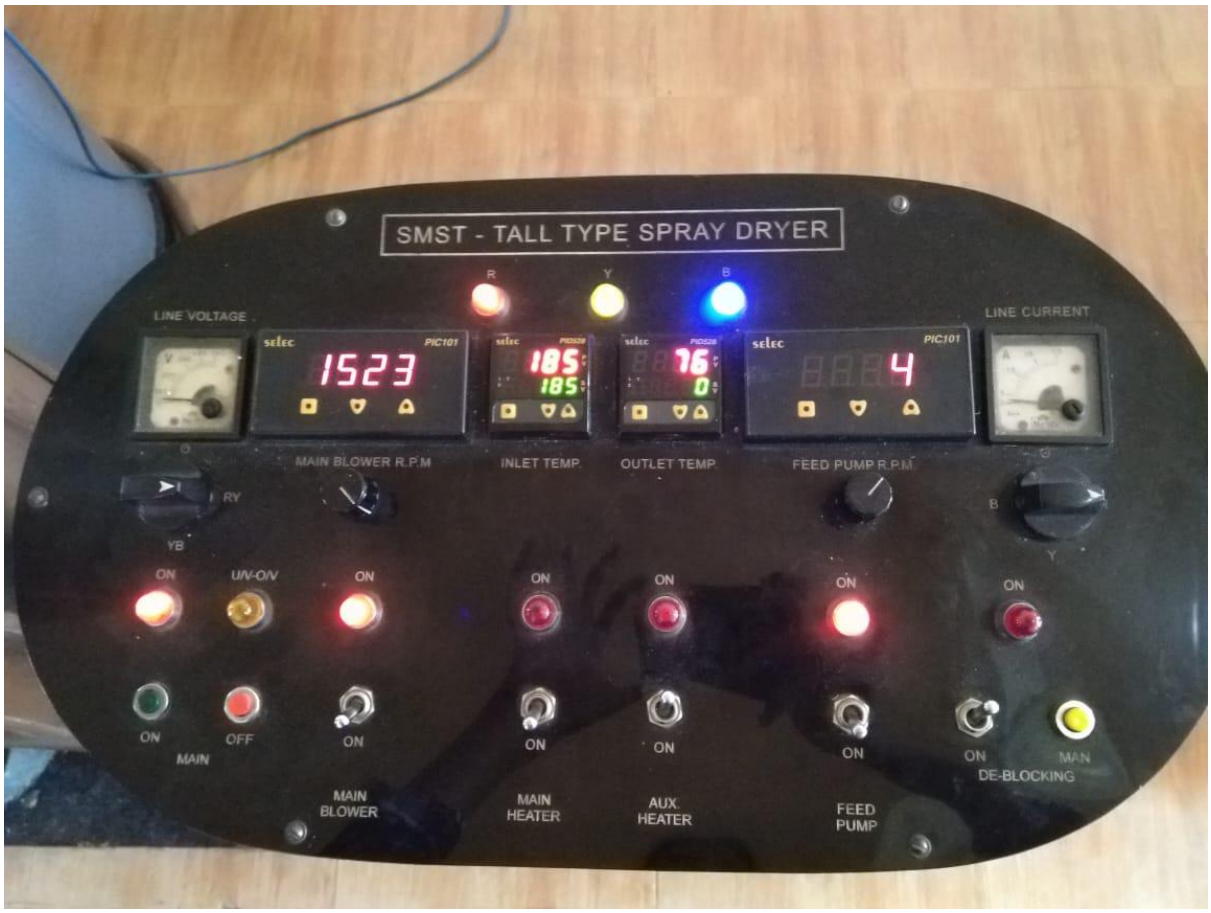


Fig3.01: Spray dryer control panel

Fig3.02: Spray dryer

3.9Analysis of encapsulated powder

3.9.1Quantitative analysis

The spray dried powder was further quantitatively analyzed for phenol, flavonoid and alkaloid in order to identify their quantity.

3.9.1.1Total Phenol Content Determination:

The phenols were determined by slightly modified Folin and Ciocalteu method. Briefly, to the 200µl of the sample extract, 800 µl of Folin Ciocalteu reagent mixture and 2 ml of 7.5% sodium carbonate added. The total content is diluted to 7 volumes with distilled water and finally kept the tubes for 2 hrs incubation in dark. The absorbance was measured at 760 nm. Gallic acid dilutions were used as standard solutions. The results of phenols are expressed in terms of Gallic acid in mg/ml of extract.

3.9.1.2 Total Alkaloid Content Determination:

A part of extract residue was dissolved in 2N HCL and then filtered. 1 ml of this solution was transferred to separatory funnel and washed with 10 ml chloroform (3 times). The pH of this solution was adjusted to neutral with 0.1 N NaOH. Then 5 ml of BCG solution and 5 ml of phosphate buffer were added to this solution. The mixture was shaken and complex extracted with 1, 2, 3- and 4-ml chloroform by vigorous shaking, the extract was then collected in a 10 ml volumetric flask and diluted with chloroform.

3.9.1.3 Total Flavonoid content:

Total flavonoid content was measured by the Aluminium chloride colorimetric assay. An aliquot (1ml) of extracts and standard solution of Catechin(100mg/ml) was added to 10ml volumetric flask containing 4ml of distilled water. To this 0.3ml 5% NaNO2 was added. After 5 minutes 0.3 ml of 10% $AlCl_3$ was added. Then after 1 min, 2 ml of 1M NaOH was added and total volume was made up to 10ml with distilled water. The solution was mixed well and absorbance was measured against prepared reagent blank at 510nm. Total flavonoid content of leaf extract expressed as milligram catechin equivalence (CE)/100G fresh weights. All samples were analysed in triplicates.

3.9.2. Physio chemical properties of microencapsulated papaya leaf powder

Papaya leaf powder samples were analyzed for physicochemical properties described in AOAC and from the literature collected.

3.9.2.1Total soluble solids

Total soluble solids (TSS) in fresh papaya leaf juice and the diluted papaya leaf juice powder was measured using hand refractometer (Erma inc, Tokyo). Papaya leaf juice powder was mixed with water and allows the sample to settle. One or two drops of the prepared sample were placed on the hand refractometer for TSS measurement. It was expressed in degree Brix (AOAC,1990).

3.9.2.2 pH

The pH of the reconstituted papaya leaf juice powder was measured using a digital pH meter (Systonics, Naroda) available at Dept. FAPE, Tavanur. Distilled water of pH 7 was used to calibrate the pH meter before determination of pH of the powder. Ten millilitres of diluted powder sample were taken in a beaker and the electrode of pH meter was immersed in the sample to determine pH. The reading was directly recorded from the pH meter and was repeated thrice for precision. Average value was considered as the pH of the powder (AOAC,1990).

3.9.2.3 Water activity

The water activity of the papaya leaf powder was carried out using Qua lab water activity meter (M/s. Aqua Lad, USA; model series 3TE). Water activity (a_w) is a measure of the amount of water available (Troller and Christian, 1978). For determining the water activity, the papaya leaf powder was filled in the disposable cups of the water activity meter and the sample drawer knob is turned to OPEN position. After opening the drawer, the disposable cup with powder was then placed in the drawer and closed. The sample drawer knob was then turned to the READ position and the water activity of the powder was noted from the LCD display of the water activity meter. Experiment repeated three times and the average value taken as the water activity.

3.9.2.4 Bulk Density

The bulk density of spray dried powder was measured according to the procedure described by GONG *et al.,* (2008) and Lebrun *et al.,* (2012). Approximately, one gram of the powder was freely poured into a 10ml graduated cylinder without tapping. The level of samples

in cylinder was noted for measuring loose bulk density of spray dried powder. Same sample was repeatedly tapped manually by lifting and dropping the cylinder under its own weight at a vertical distance. This was done until negligible difference in volume between succeeding measurements was observed and is used for the measurement of tapped bulk density of powder. Experiment was repeated for accuracy and average values were considered as loose bulk density and tapped bulk density of samples. The bulk density of powder was computed using the following expression

$$\text{Loose bulk density(g/ml)} = \frac{\text{Weight of sample (g)}}{\text{Bulk sample volume(ml)}} \quad \ldots\ldots\ldots(3.1)$$

$$\text{Tapped bulk density(g/cm}^3\text{)} = \frac{\text{Weight of sample (g)}}{\text{Tapped powder volume (cm}^3\text{)}} \quad \ldots\ldots.(3.2)$$

3.9.2.5 Colour characteristics

Colour of product is an important parameter that will be valued during product marketing. Colour of the papaya leaf juice powder-based nutraceutical powders were measured using Hunter lab colour flex meter (Hunter Associates Laboratory, Reston, Virgina, USA). The colour was measured by using CIELAB scale at 10^0 observer at D_{65} illuminant. It works on the principle of focusing the light and measuring the energy reflected from the sample across the entire visible spectrum. The three-dimensional scale L*, a* and b* values were used for colour measurement. The luminance (L*) forms the vertical axis, which indicates light-dark spectrum with a range from 0(black) to 100(white). In the same way, a* indicates the green – red spectrum with a range of -60(green) to +60(red) and b* indicates the blue-yellow spectrum with a range from -60(blue) to +60(yellow)dimensions respectively (reddy *et al;* 2014).

The instrument was standardised before placing the sample by placing black and white tile provided with the instrument. Once the instrument was standardised, it was ready to measure the colour. It can also be cross checked by placing the white tile which was provided by the L*, a* and b* values. The samples were filled in the sample cup. The deviation of the colour of the sample to standard was also observed and recorded in the computer interface. The experiment was repeated thrice for each sample and average was taken as colour range.

3.10 Reconstitution properties of spray dried papaya leaf powder

Reconstituted properties of food powders are important for its market quality and consumer acceptability. Reconstitution properties such as solubility, wettability and water solubility index of spray dried papaya leaf juice powder was determined using standard procedures as explained below.

3.10.1 Wettability

Time in seconds to achieve complete wetting of the papaya leaf powder when it is poured into water at room temperature is noted as wettability. For wettability determination, a glass funnel held on a stand and was set over the breaker containing 100ml of distilled water at room temperature. A glass rod was kept inside the funnel to block its lower opening. To this setup, one-gram sample was placed around the glass rod was lifted. The time taken for complete wetting of the powder particles were noted using a stop watch. Determination of wettability was carried out thrice for spray dried powder and the average value was considered wettability of powder (Jinapong *et al*., 2008, Desousa *et al., 2008,* Falade and Omojola 2010).

3.10.2 Solubility

Solubility of papaya leaf powder was carried by the method explained by Chauca *et al. (2005).* One-gram papaya powder was mixed with 100ml of water at room temperature for 30 min. A 10ml aliquot of the supernatant solution was transferred to a 15 ml centrifuge tube and centrifuged for 15 min at 15,000 rpm. The aliquot of the supernatant was then taken in a pre-weighed aluminium moisture dish, evaporated on a steam bath and dried in an oven at 110℃ overnight. The solubility was calculated as per equation

$$\text{Solubility (\%)} = \frac{10 \times \text{Solid in supernatant(g)}}{\text{Sample weight(g)}} \times 100 \qquad \ldots\ldots(3.3)$$

3.10.3 Water solubility index

The water solubility index of papaya leaf powder was determined by mixing 2.5g powder sample and distilled water (30ml) vigorously in 100ml centrifuge tube, incubated in a 37℃-water bath for 30 min and then centrifuged for 20 min at 10000 rpm in a centrifuge (Rotck, 50Cps). The supernatant was carefully collected in a pre-weighed beaker and oven dried at a temperature of 103±2℃ (Anderson,1969 and Sabhadinde ,2014). Water solubility index was calculated as follows

$$\text{Water solubility index (\%)} = \frac{\text{Weight of supernatant}}{\text{Weight of sample}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots (3.4)$$

3.10.4 Flowability and Cohesiveness

The flowability and cohesiveness of a powder can be measured using Carr's index (CI) and Hunsner ratio (HR).

3.10.4.1Carr's index (CI)

The compressibility index or the Carr's index can be measured from the pre-determined bulk density and tapped bulk density values. The index of papaya leaf juice powder was determined by equation below and its powder characteristics was determined by referring the values by Lebrun *et al,* (2012)

$$\text{Carr's index (\%)} = \frac{\text{Tapped bulk density(g/ml)} - \text{Loose bulk density (g/ml)}}{\text{Tapped bulk density(g/ml)}}$$

3.10.4.2 Hunsner ratio (HR)

Hausner ratio of the papaya leaf juice powder was determined by the method described by Shisir *et al,(2015).* HR value for the powder was calculated by the formula given by:

$$\text{Hausner ratio (HR)} = \frac{\text{Tapped bulk density (g/ml)}}{\text{Loose bulk density (g/ml)}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.5)$$

Sl no	Flowability	Carr's Index	Hausner Ratio
1	Excellent	0-10	1-1.11
2	Good	11-15	1.12-1.18
3	Fair	16-20	1.19-1.25
4	Possible	21-25	1.26-1.34
5	Poor	26-31	1.35-1.45
6	Very poor	32-37	1.46-1.59
7	Very'very poor	>38	>1.60

Table 3.03: Specifications for Carr's Index and Hausner Ratio

Chapter 4

RESULTS AND DISCUSSIONS

4.1 Qualitative Analysis

Preliminary Qualitative analysis results of crude C. papaya leaf are mentioned in **Table 4.1** which revealed the presence of components including alkaloid, phenol, flavonoid etc.

	Cold extraction			**Hot extraction**		
	Mature	Intermediate	Tender	Mature	Intermediate	Tender
Flavonoids	+	+	+	+	+	+
Saponins	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Phenols	+	+	+	+	+	+
Proteins	+	+	+	+	+	+
Cardiac glycosides	-	-	-	-	-	-
Terpenoids	+	+	+	s	S	-
Carbohydrates	+	+	+	+	+	+

Keys : Presence: +, Slight presence: S, Absence:

Table 4.01: Result of Qualitative analysis of water extract of *C. papaya* leaf

4.2 Quantitative Analysis

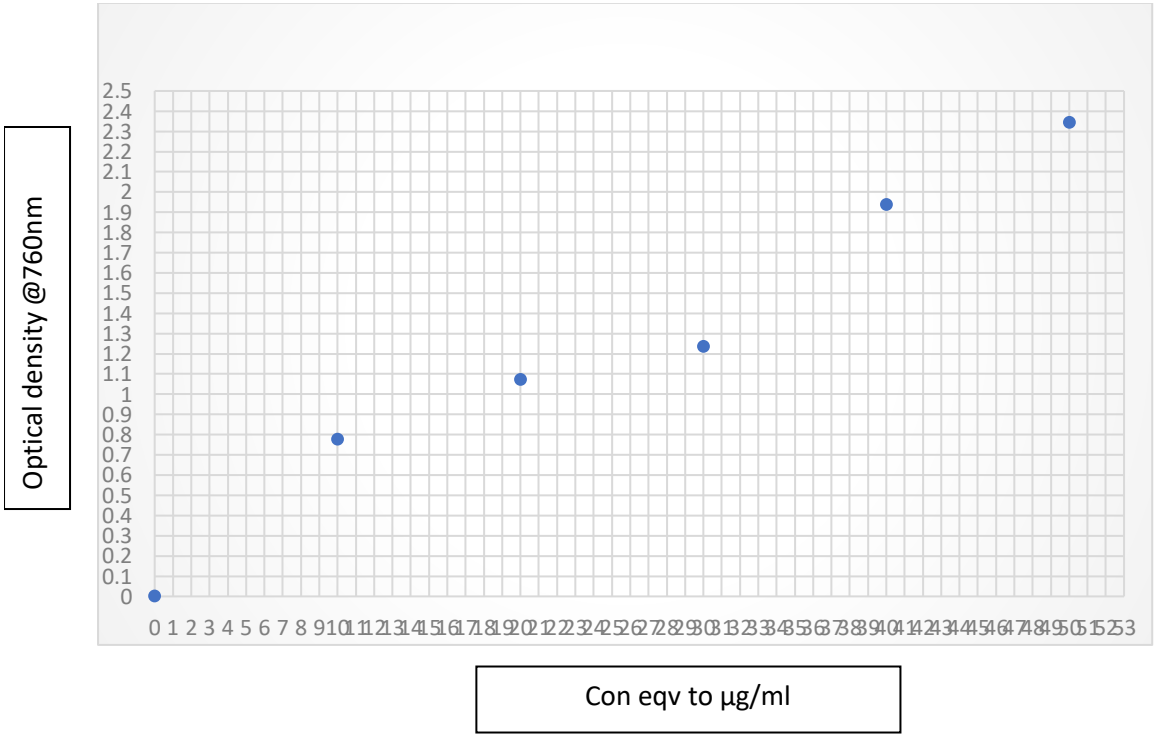
Quantitative analysis results of C. papaya leaf extract are mentioned in **Table 4.2** which revealed the content of components including alkaloid, phenol, flavonoid etc..

4.2.1 Total phenol content

Total phenols contents were determined from the linear equation of a standard curve prepared with Gallic acid**.** The content of total phenolic compound expressed as mg /g Gallic acid equivalent (GAE) of dry extract.

Sample	Volume of Gallic acid	Volume of distilled water	Gallic acid(g/ml)	Gallic acid concentration (µg/ml)	OD value
Blank	0	1	0.00	0	0
S1	0.2	0.8	0.01	10	0.776
S2	0.4	0.6	0.02	20	1.071
S3	0.6	0.4	0.03	30	1.237
S4	0.8	0.2	0.04	40	1.938
S5	1	0	0.05	50	2.345

Table 4.02: Standard values for Gallic acid



Graph 4. 1: Standard curve of Gallic acid

Sample	Wt/ml(µg/ml)	Wt/200ml(mg/200ml)
Hot tender	29.75	5.85
Hot intermediate	29	5.8
Hot mature	29.5	5.85
Cold tender	27.75	5.55
Cold intermediate	28.25	5.6
Cold mature	25.5	5.1
Juice Tender	12.75	2.55
Juice Intermediate	15	3
Juice mature	18	3.6

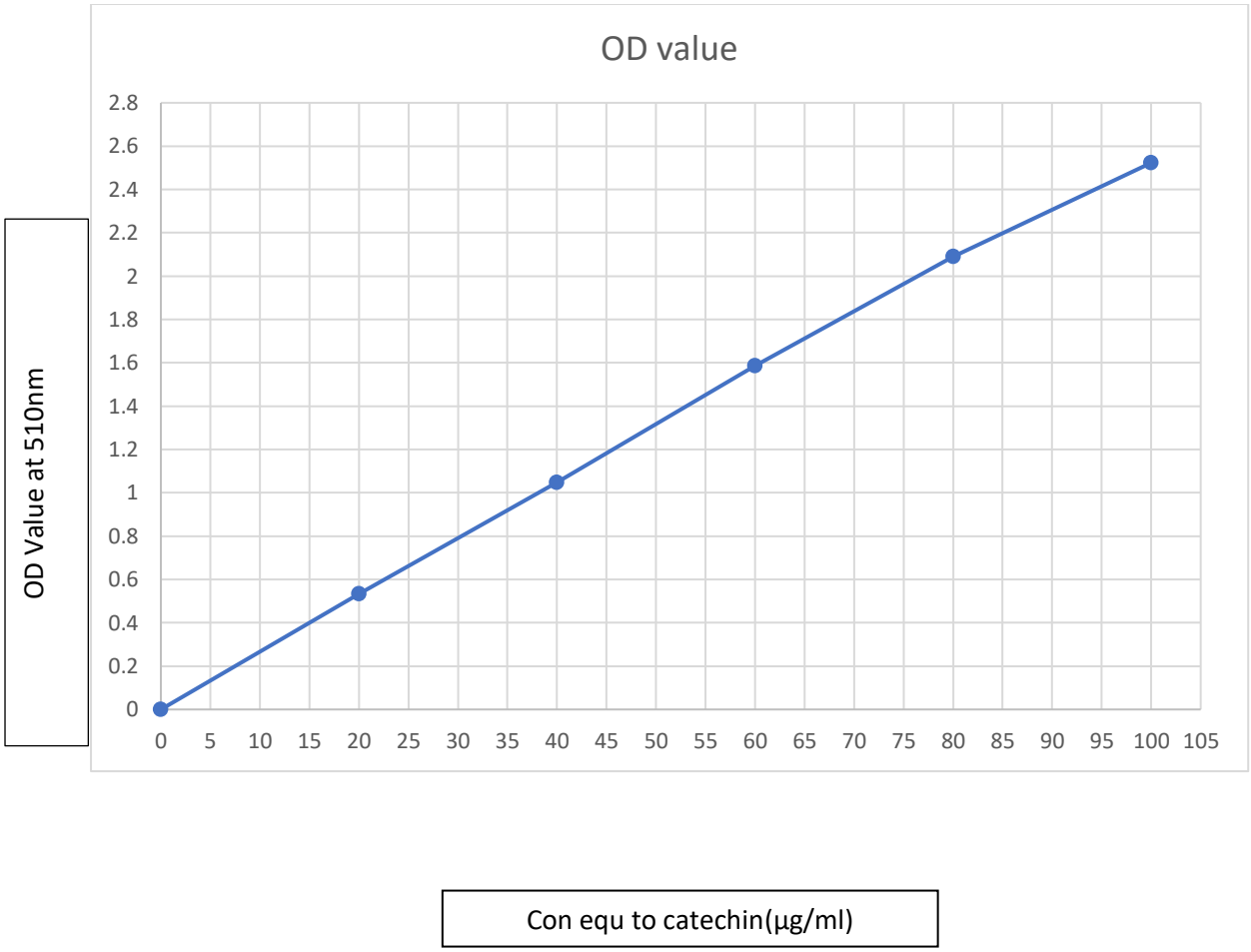
Table4.03: Total phenol content in different leaf sample

4.2.2. Total Flavonoid content

Total flavonoid contents were determined from the linear equation of a standard curve prepared with Catechin**.** The content of total flavonoid compound expressed as mg /ml catechin equivalent (CE) of dry extract.

Sample	Volume of catechin	Volume of distilled water	Catechin (mg/ml)	Catechin concentration (µg/ml)	OD value
Blank	0	1	0	0	0
S1	0.2	0.8	0.02	20	0.533
S2	0.4	0.6	0.04	40	1.048
S3	0.6	0.4	0.06	60	1.048
S4	0.8	0.2	0.08	80	1.586
S5	1	0	0.1	100	2.523

Table4.04: Standard values in catechin curve



Graph4.02: Standard curve of catechin

Sample	Wt/ml(µg/ml)	Wt/200ml(mg/200ml)
Hot tender	1.4	0.28
Hot intermediate	2.0	0.4
Hot mature	2.4	0.48
Cold tender	2.2	0.44
Cold intermediate	3.6	0.72
Cold mature	2.6	0.52
Juice Tender	1.3	0.26
Juice Intermediate	1.8	0.36
Juice mature	3.0	0.6

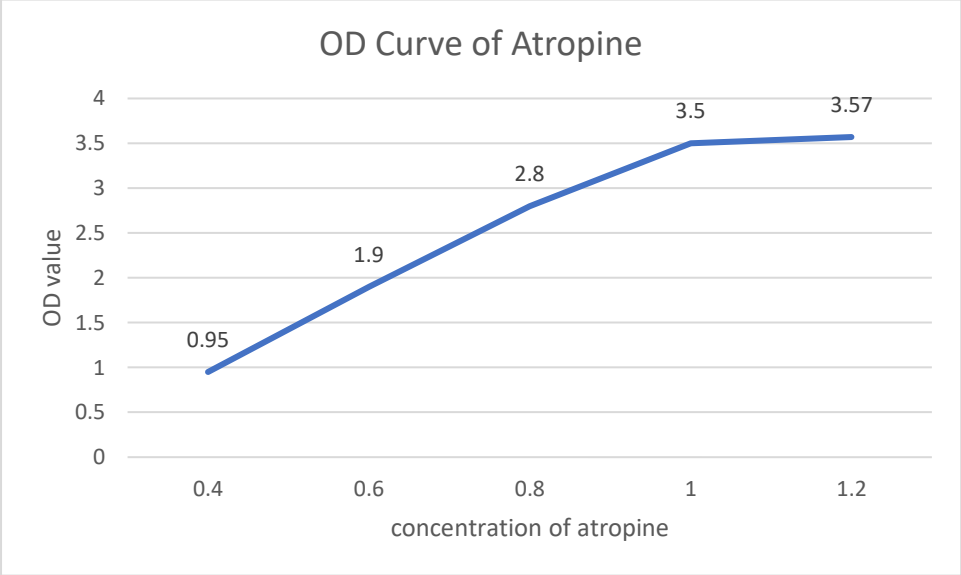
Table4.5: Total flavonoid content in different leaf sample

4.2.3 Total alkaloid content

Presence of alkaloid was tested quantitatively by UV spectrometer by using atropine as standard. This method is based on the reaction between alkaloid and bromocresol green (BCG.

Sample	Volume of atropine	Volume of distilled water	Atropine (mg/ml)	Atropine concentration (µ9/ml)	OD value
Blank	0	1	0	0	0
S1	0.2	0.8	0.02	20	.95
S2	0.4	0.6	0.04	40	1.9
S3	0.6	0.4	0.06	60	2.8
S4	0.8	0.2	0.08	80	3.5
S5	1	0	0.1	100	3.57

Table4.06: Standard curve value of Atropine



Graph 4.03: Standard curve of Atropine

Sample	Wt/ml(µg/ml)	Wt/200ml(mg/200ml)
Hot tender	2.0	0.4
Hot intermediate	2.44	0.488
Hot mature	3.69	0.738
Cold tender	2.88	0.576
Cold intermediate	3.25	0.65
Cold mature	2.01	0.402
Juice Tender	2.01	0.402
Juice Intermediate	2.5	0.5
Juice mature	2.44	0.488

Table4.07: Total alkaloid content in different leaf sample

4.3Analysis of encapsulated powder

4.3.1 Quantitative analysis

4.3.1.1 Total Phenol Content Determination:

After the analysis and further calculation, it was found that the phenol content in the spray dried papaya leaf juice is more over similar to the fresh juice. It has a concentration of 25.5 Wt/ml(µg/ml) there is a slight difference from that of the value of fresh juice.

4.3.1.2 Total Alkaloid Content Determination:

After the analysis and further calculation, it was found that the alkaloid content in the spray dried papaya leaf juice is similar to that of the fresh juice. It has a concentration of 0.36 Wt/ml(µg/ml).

4.3.1.3 Total Flavonoid content:

After the analysis and further calculation, it was found that the flavanoid content in the spray dried papaya leaf juice is similar to that of the fresh juice. It has a concentration of 3.25Wt/ml(µg/ml). The overall view of quantitative analysis of papaya leaf juice powder is as follows:

Component analysed	OD value	Wt/ml(µg/ml)	Wt/200ml(mg/200ml)
Phenol	2.248	25.5	5.1
Flavonoid	0.36	0.36	0.72
Alkaloid	1.470	3.25	0.65

Table4.08: Overall content of encapsulated powder

4.3.2 Physico chemical properties of microencapsulated papaya leaf powder

4.3.2.1 Total soluble solids

Total soluble solids (TSS) in fresh papaya leaf juice and the diluted papaya leaf juice powder was measured using hand refractometer (Erma inc, Tokyo). The TSS of fresh papaya leaf juice is found to be 7°Brix and that of diluted papaya leaf juice powder is found to be 6° Brix.

4.3.2.2 pH

The pH of the reconstituted papaya leaf juice powder was measured using a digital pH meter (Systonics, Naroda) available at Dept. FAPE, Tavanur. Distilled water of pH 7 was used to calibrate the pH meter before determination of pH of the powder. Average value was considered as the pH of the powder (AOAC,1990). The pH of reconstituted papaya leaf juice powder was found to be 5.18 (acidic in nature).

4.3.2.3 Water activity

The water activity of the papaya leaf powder was carried out using Qua lab water activity meter (M/s. Aqua Lad, USA; model series 3TE). Water activity(a_w) is a measure of the amount of water available (Troller and Christian, 1978). Experiment repeated three times and the average value taken as the water activity. The water activity of papaya leaf juice powder was found to be 0.373 at 38.5°C.

4.3.2.3 Bulk Density

The bulk density of spray dried powder was measured according to the procedure described by GONG *et al.* (2008) and Lebrun *et al* (2012). The Bulk sample volume(ml) was found to be 3.43 ml and therefore the loose bulk density is found to be 0.3(g/ml).The Tapped powder volume (cm^3) was found to be 2.2 cm^3 , therefore the tapped bulk density is found to be 0.4545(g/cm^3).

4.3.2.5 Colour characteristics

Colour of product is an important parameter that will be valued during product marketing. Colour of the papaya leaf juice powder-based nutraceutical powders were measured by Hunter lab colour flex meter (Hunter Associates Laboratory, Reston, Virgina, USA).

L*=63.12

a*=0.09

b*=5.17

4.4 Reconstitution properties of spray dried papaya leaf powder

Reconstituted properties of food powders are important for its market quality and consumer acceptability. Reconstitution properties such as solubility, wettability and water

solubility index of spray dried papaya leaf juice powder was determined using standard procedures as explained below.

4.4.1 Wettability

Time in seconds to achieve complete wetting of the papaya leaf powder when it is poured into water at room temperature is noted as wettability. It was found to be 205 seconds.

4.4.2 Solubility

Solubility of papaya leaf powder was carried by the method explained by Chauca etal.*(2005).*The amount of Solid in supernatant (g) was found to be 0.075g and solubility was found to be 75%.

4.4.3 Water solubility index

The Weight of supernatant (g) was found to be 1.25g and the water solubility index was 50%.

4.4.4 Flowability and Cohesiveness

4.4.4.1 Carr's index (CI)

The index of papaya leaf juice powder was determined by equation below and its powder characteristics was determined by referring the values by Lebrun *et al,*(2012).

It was found to be 51.5% and it is above 38% and the flowability was found to be very poor.

4.4.4.2 Hunsner ratio (HR)

Hausner ratio of the papaya leaf juice powder was determined by the method described by Shisir *et al,(2015).*

It was found to be 1.515 and it is in between 1.46-1.59 and the flowability was found to be very poor.

CHAPTER 5
SUMMARY AND CONCLUSION

Carica papaya is one of the valuable plants used for various purposes in medicinal field. The whole *C. papaya* i.e., fruits, seeds, bark and leaves are used for treatment and curing of many diseases. Papaya leaf contain many active components that can increase the total antioxidant power in blood and reduce lipid peroxidation level such as papain, chymo-papain, tocopherol, flavonoids etc. Studies have found that the powder from papaya leaf has substances responsible for the release and production of thrombocytes or platetes and have shown effective in treating dengue viral infections. Hence it could be used as a complimentary drug in dengue fever. Papaya fruit juice and leaf extract have demonstrated anticancer property. In addition to cancer fighting substances, papaya leaves contain larger doses of important nutrients that support the immune system including Vitamin A, C, and E. Most importantly it contains vitamin B17 used in traditional chemotharapine.

The use of these plants as a supplement in food, taking in to account that these plants can present a significant amount of useful components.

The work started with the grading of collected papaya leaves into tender, intermediate and mature according to maturity stages based on parameters such as size, length, color etc. Then the water-soluble components of the leaves were extracted using distilled water through methods of hot extraction, cold extraction and fresh juice prepared using electronic mixer for each of the maturity samples. The qualitative analysis of all the extract sample indicated the presence of alkaloids, phenols, flavonoids, antioxidants, saponins, carbohydrates, tannins and terpenoids. The quantitative analysis of alkaloids, phenols, flavonoids and antioxidants were carried out for each sample combinations. And it was found that the sample combination of intermediate maturity leaves extracted through cold extraction method shown the best results for alkaloids, phenols, flavonoids and antioxidants than other samples.

The superior sample was then micro encapsulated using maltodextrin (15% wt./wt.) as wall material through spray technology. spray drying is the most common, economical and commercial method for microencapsulation process. Spray drying is a simple, fast and continuous process in which a liquid or paste is transformed in a powder of micro particles after a relatively short drying period and can be used for the heat liable material because of the low temperature that the wall material reaches. Also spray drying removed the unfavourable colour and odour of the extract giving fine powder of good acceptance.

Again, the qualitative and quantitative analysis spray dried powder was carried out. And it was found that there is no change in flavonoid and alkaloid content, while only a slight decrease in phenol content were found.

After that the physic-chemical and reconstitution properties of the spray dried product were analyzed giving that they have very good compatibility towards food grades. The microencapsulated papaya leaf powder enriched different beverage, salad dressing was developed. And the sensory evaluation of enriched food is carried out giving good acceptance for grape and lime flavoured RTS beverage.

The following are the suggestions for future research work on the microencapsulation of papaya leaf juice powder extract.

1. Studies may be carried out with different combinations of wall material for the encapsulation to improve the stability of the encapsulated powder.
2. Studies may be carried out using different drying methods for the encapsulation.

3.Studies may be carried for different papaya leaf enriched food combinations

REFERENCE

Adeolu Alex A, Vivian Eguonor O. antinociceptive and anti-inflammatory studies of the aqueous leaf extract of Carica papaya in laboratory animals. Asian J Exp Biol Sci 2013; 4: 89-96.

Ahmad n, Fazal H, Ayas M, Abbasi BH, Mohammed I, Fazal.. Dengue fever treatment with Carica papaya leaves extracts. Asian Pac J Trop Biomed 2011;1(4): 330-3.

Altemimi, A.W.; Watson, D.G.; Kinsel, M.; Lightfoot, D.A. Simultaneous extraction, optimization, and analysis of flavonoids and polyphenols from peach and pumpkin extracts using a tlc-densitometric method. Chem. Cent. J. 2015, 9, 1–15.

Ammar Altemimi, Naoufal Lakhssassi, Azam Baharlouei, Dennis G. Watson, and David A. Lightfoot. 2015. Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts doi: 10.3390/plants6040042.

Anandaraman, S. and Reineccius, G.A. 1986. Stability of encapsulated orange peel oil. Food. Technol. 40: 88-93.

Augustin, M.A., Sanguansri, L., Margetts, C., and Young, B. 2001. Microencapsulation of food ingredients. Food. Aust. 53: 220-223.

Azwanida NN (2015) A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation. Med Aromat Plants 4:196. doi:10.4172/2167-0412.1000196

Balasubramani, P., Viswanathan, R., and Vairamani, M. 2013. Response surface optimisation of process variables for microencapsulation of garlic (Allium sativum L.) oleoresin by spray drying. Biosyst. Eng. 114: 205- 213.

Baskaran C, Ratha Bai V, Velu S , Kumaran K. The efficacy of Carica papaya leaf extract on some bacterial and a fungal strain by diffusion method. Asian Pac J Trop Biomed 2012: 2(Suppl2): S658-62.

Belghith, H., Chaabouni, S. E., and Gargouri, A. 2001. Stabilization of Penicillium occitanis cellulases by spray drying in presence of Maltodextrin. Enzym. Microb. Technol. 28: 253-258.

Buffo, R.A. and Reineccius, G.A. 2000. Optimization of gum acacia/modified starches/ maltodextrin blends for the spray drying of flavours. Perfumer. Flavorist. 25: 37-49.

Cai, Y.Z. and Corke, V. 2000. Production and properties of spray- dried Amarnthus Betacyanin Pigments. J. Food Sci. 65(6): 1248-1252.

Celestine Uzoma Aguoru, Christopher Ogbodo, Joseph Olalekan Olasan Chemosystematics Studies on Six Varieties of Mangifera indica L. **Agricultural Sciences**, Vol.8 No.3, March 27, 2017

Cowan, M.M. 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev. 564-582.

Criagg, G.M., David, J.N. 2001. Natural product drug discovery in the next millennium. J. Pharm. Biol., 39: 8-17.

Deis, R.C. 1997. Spray drying - innovative use of an old process. Food Prod. Des. 7(2): 136-148.

Desai, K.G.H. and Park, H.J. 2005. Recent developments in microencapsulation of food ingredients. Dry. Technol. 23: 1361-1394.

Desobry, S., Netto, F.M., and Labuza, T.P. 1997. Comparison of spray-drying, drum-drying and freeze-drying for β-carotene encapsulation and preservation. J. Food Sci. 62: 1158-1162.

Edoga, H.O., Okwu, D.E., Mbaebie, B.O. 2005. Phytochemicals constituents of some Nigerian medicinal plants. Afr. J. Biotechnol., 4(7): 685-688.

Ersus, S. and Yurdagel, U. 2007. Microencapsulation of anthocyanin pigments of black carrot by spray drier. J. Food Eng. 80: 805-812.

Fernandes, L. P., Turatti, I.C., Lopes, N.P., Ferreria, J C., and Oliveira, W.P. 2008. Volatile retention and antifungal properties of spray-dried microparticles of Lippia sidoides essential oil. Dry. Technol. 26(12): 1534-1542.

Fernandes, R.V.B., Borges, S.V., and Botrel, D.A. 2014. Gum arabic/starch/ maltodextrin/inulin as wall materials on the microencapsulation of rosemary essential oil. Carbohyd. Polym. 101: 524-532.

Gharsallaoui, A., Roudaut, G., Chambin, O., Voilley, A., and Saurel, R. 2007. Applications of spray drying in microencapsulation of food ingredients: an overview. Food Res. Int. 40(9): 1107-1121.

Gibbs, B.F., Kermasha, S., Ali, I., and Mulligan, C.N. 1999. Encapsulation in the food

Ikeyi Adachukwu , Ogbonna Ann and Eze Faith U. (2013). Phytochemical analysis of paw-paw (carica papaya) leaves. Int. J. LifeSc. Bt & Pharm. Res.

Ikeyi Adachukwu P, Ogbonna Ann O, and Eze Faith U, "Phytochemical Analysis of Paw-Paw (Carica papaya) Leaves," International Journal of Life Sciences Biotechnology and Pharma Research, Vol. 2, No. 3, pp. 347-351, July 2013.

Isela E. Juárez-Rojop,Carlos A.Tovilla-Zárate,Dora E. Aguilar-Domínguez ,Luis F. Roa-de la Fuente,Carlos E. Lobato-García ,Jorge L. Blé-Castillo ,Leonor López-Mera ,Juan C. Díaz-Zagoya,Deysi Y. Bermúdez-Ocaña. (2014). Phytochemical screening and hypoglycemic activity of *Carica papaya* leaf in streptozotocin-induced diabetic rats. Rev. bras. farmacogn. vol.24 no.3 Curitiba.ISSN: 0970-020 X

Jain, P., Arora, S., and Rai, T. 1997. Flavour encapsulation and its application. Beverage Food World. 24(4): 21-24.

James Hamuel Doughari Phytochemicals: Extaction methods,basic structures and mode of action as potential chemotherapeutic agents. 2010.

JG de Oliveira, Jurandi Gonçalves de Oliveira a , Angela Pierre Vitória (2011). Papaya: Nutritional and pharmacological characterization, and quality loss due to physiological disorders. J. Food Research International 44 1306–1313.

Julianti T, DE Mieri M, Zimmermann S, Ebrahami SN, Kaiser M, Neuburger M, et al. HPLC-Based activity profiling for anti-plasmodial compounds in the traditional Indonesian medicinal plant Carica papaya L. J Ethnopharmacol 2012; 144: 453-6.

Julianti T, Outfir M, Hamburger M, Quantification of the anti-plasmodial alkaloid carpaine in papaya (Carica papaya) leaves. Planta Med 2014; 80:1138-42.

Julianti T, Oufir M, Hamburger M. Quantification of the antiplasmodial alkaloid carpaine in papaya (Carica papaya) leaves.pp 1138

Kenyon, M.M. and Anderson, R.J. 1988. Maltodextrin and low dextrose equivalence corn syrup solids in flavour encapsulation. In: Risch, S.J. and Reinceccius, G.A. (eds), Flavor encapsulation. American Chemical Society, Washington, pp.7-11.

Kha, T.C., Nguyen, M.H., and Roach, P.D. 2010. Effects of spray drying conditions on the physicochemical and antioxidant properties of the Gac (Momordica cochinchinensis) fruit aril powder. J. Food Eng. 98: 385– 392.

Koehn, F.E., Carter, G.T., 2005. The evolving role of natural products in drug discovery. Nat. Rev. Drug Discov. 4, 206-220.

Lezama, G.A.Y., Alvarez, D.L., Flores, J.M.E., Alonso, P.C., Niranjan, K., López, G.G.F., and Beltrán, A.L. 2012. Preparation and characterization of nonaqueous extracts from chilli (Capsicum annuum L.) and their microencapsulates obtained by spray-drying. J. Food Eng. 112: 29-37.

Liew SY, Stanbridge EJ, Yousoff K, Shafee N. Hypoxia affects cellular responses to plant extracts. J Ethnopharmacol 2012:144: 453-6.

Lin, C.C., Lin, S.Y., and Hwang, L.S. 1995. Microencapsulation of squid oil with hydrophilic macromolecules for oxidative and thermal stabilization. J. Food Sci. 60: 36-39.

Lorato Lekgari. (2010). Extraction of phyyochemicals. Biotech articles McKernan, W.M. 1992. Microencapsulation in the flavor industry. Flavour Ind. 4: 72-78.

Monika Gupta, Shweta Thakur, Anuradha Sharma and Sudhakar Gupta (2013). Qualitative and quantitative analysis of phytochemicals and pharmacological value of some dye yielding medicinal plants. Oriental Journal Of Chemistry.

Moussaoui A. El., et al., Cellular and Molecular Life Sciences 58(4) (2001) 556-570.

Mutalik G. S., Research Needs and Traditional Medicine in South East Asia Region – Background paper No. SEA/RPD/Tradmed/RSG Meet 1/2, WHO office, N. Delhi 1972.

Nguyen YY, Parat MO, Shaw PN, Hewavitharana AK, Hodson MP, Traditional aboriginal preparation alters the chemical profile of Carica papaya leaves and impacts on cytotoxity towards human squamous cell carcinoma. PLoS One 2016; 11(2): e0147956.

Nisar Ahmad, Hina Fazal, Muhammad Ayaz, Bilal Haider Abbasi, Ijaz Mohammad,and Lubna Fazal Dengue fever treatment with *Carica papaya* leaves extracts Asian Pac J Trop Biomed. 2011 Aug; 1(4): 330–333 doi: 10.1016/S2221-1691(11)60055-5.

Noriko Otsuki , Nam H. Dang , Emi Kumagai , Akira Kondo, Satoshi Iwata, Chikao Morimoto (2009). Aqueous extract of Carica papaya leaves exhibits anti-tumor activity and immunomodulatory effects. J. Ethnopharmacology 127 (2010) 760–767.

Noriko Otsuki , Nam H. Dang , Emi Kumagai , Akira Kondoc, Satoshi Iwataa, Chikao 2010 Morimotoa Aqueous extract of Carica papaya leaves exhibits anti-tumor activity and immunomodulatory effects Journal of Ethnopharmacology 127 (2010) 760–767.

occurring sources such as plants, insects, animals

Okoko T, Ere D. Reduction of hydrogen peroxide- induced erythrocyte damage by Carica papaya laf extrac. Asian Pac J Trop Biomed 2012; 2(6): 449-53.

Okwu, D.E., Okwu, M.E. 2004. Chemical composition of Spondias mombin linn. plant parts. J. Sustain. Agric. Environ., 6(2): 140-147 ORIENTAL JOURNAL OF CHEMISTRY

Parekh, J., Chanda, S. 2008. Phytochemicals screening of some plants from western region of India. Plant Arch., 8: 657662.

Parize, A.L., Souza, T.C.R., Favere, I.M.V., Laranjeira, M.C.M., Spinelli, V., and Longo, E. 2008. Microencapsulation of the natural urucum pigment with chitosan by spray drying in different solvents. Afr. J. Biotechnol. 7(17): 3107-3114.

Parle Milind et.al. (2011). Basketfull benefits of papaya International research journal of pharmacy pp 6-12.

Patil, P.S. & Shettigar, R. (2010). An advancement of analytical techniques in herbal research J. Adv. Sci. Res. 1(1); 08-14.

Perry L. M., J. Metzger, (1980). Medicinal Plants of East and Southeast Asia: Attributed Properties and Uses. Cambridge: The MIT Press.Pg. 475-481

Prabhavathi R. M. , Prasad M. P and Jayaramu M. (2016). Studies on Qualitative and Quantitative Phytochemical Analysis of Cissus quadrangularis. Advances in Applied Science Research, 2016, 7(4):11-17.

Raja, K.C.M., Sankarikutty, B., Sreekumar, M., Jayalekshmy, A., and Narayanan, C.S. 1989. Material characterization studies of maltodextrin samples for the use of wall material. Starch/ starke. 41: 298-303.

Reineccius, G.A. 1988. Spray-drying of food flavors. In: Reineccius, G.A. and Risch, S.J. (eds), Flavor encapsulation. American Chemical Society, Washington, pp.55-66.

Rosenberg, M., Kopelman, I.J., and Talman, Y. 1985. A scanning electron microscopy study of microencapsulation. J. Food Sci. 50:130-144

Sangian H, Faramarzi H, Yazdinezhad A, Mousavi SJ, Zamani Z, Noubarani M, Ramazani A. Antiplasmodial activity of ethanolic extracts of some selected medicinal plants from the northwest of Iran. 2013 Nov;112(11):3697-701. doi: 10.1007/s00436-013-3555-4. Epub 2013 Aug 7.

Shahidi, F. and Han, X.Q. 1993. Encapsulation of food ingredients. Crit .Rev. Food Sci. Nutr.33: 501-547.

Shaw, L.A., McClements, D.J., and Decker, E.A. 2007. Spray-dried multilayered emulsions as a delivery method for ω-3 fatty acids into food systems. J. Agric. Food chem. 55: 3112-3119.

Sheu, T.Y. and Rosenberg, M. 1995. Microencapsules consisting of Whey proteins and Carbohydrates. J. Food Sci. Eng. Process. 63(3): 491-494.

Silva, P.I., Stringheta, P.C., Teófilo, R.F., and deOliveira, I.R.N. 2013. Parameter optimization for spray-drying microencapsulation of jaboticaba (Myrciaria jaboticaba) peel extracts using simultaneous analysis of responses. J. Food Eng. 117: 538-544.

Suhas J Vyas, Taslim T Khatri , Pragnesh N. Dave,Hitteendra S joshi 2007. Biochemical constituents in leaf of *Carica papaya* – ethnomedicinal plant of Kachchh region International Letters of Natural Sciences Vol. 12 (2014) pp 16-20

Tan SA, Ramos S, Martin MA, Mateos R, Harvey M, Ramanathan S, et al. Protective effects of papaya extracts ontert-butyl hydroperoxide mediated oxidative injury to human liver cells (an in-vitro study). Free Rad Antiox 2012; 2(3): 10-9.

Tari, T.A. and Singhal, R.S. 2002. Starch based spherical aggregates: reconfirmation of the role of amylose on the stability of a model flavouring compound, vanillin. Carbohyd. Polym. 50: 279-282.

TO Fakeye, T Oladipupo, O Showande and Y Ogunremi (2007). Effects of Coadministration of Extract of Carica papaya Linn (family Cariaceae) on Activity of Two Oral Hypoglycemic Agents. Tropical J. Pharmaceutical Research; 6 (1): 671-678.

Vasu, K., Goud, J.V., Suryam, A., Singara, Chary, M.A. 2009. Biomolecular and phytochemical analyses of three aquatic angiosperms. Afr. J. Microbiol. Res., 3(8):418-421.

Walter Last (2008). Cancer remedies. www.health-science-spirit.com/cancer6-remedies

Watanabe, Y., Fang, X., Minemoto, Y., Adachi, S., and Matsuno, R. 2002. Suppressive effect of saturated acyl L-ascorbate on the oxidation of linoleic acid encapsulated with maltodextrin or gum arabic by spraydrying. J. Agric. Food Chem. 50: 3984-3987.

WHO, 2012. Traditional medicine strategy: 2002-2005. World Health Organization press, p. 1-6.

Wilson R K, Kwan T, Kwan C Y and Sorger G J (2007), "Effect of papaya leaves Extract and Benzyl Isothiocyanate on vascular contraction'', Journal of life Science, Vol. 21, No. 10, pp. 497-507.

Wong, P.Y.Y.; Kitts, D.D. Studies on the dual antioxidant and antibacterial properties of parsley (petroselinum crispum) and cilantro (coriandrum sativum) extracts. Food Chem. 2006, 97, 505–515

Wong, P.Y.Y.Kitts, D.D. Studies on the dual antioxidant and antibacterial properties of parsley (Petroselinum crispum) and cilantro (Coriandrum sativum) extracts [2006] Food chemistry*ISSN* : 0308-8146.

Development and quality evaluation of microencapsulated powder of *C. papaya* leaf

By

MADHUJA MADHU (2015-06-011)

MALAVIKA MOHAN P (2015-06-012)

RESHMA C (2015-06-019)

THOUSEENA T N (2015-06-020)

Department of Processing and Food Engineering

KELAPPAJI COLLEGE OF AGRICULTURAL ENGINEERING AND TECHNOLOGY

TAVANUR - 679573, MALAPPURAM

INDIA

2019

Development and quality evaluation of microencapsulated powder of *C. papaya* leaf

By

MADHUJA MADHU (2015-06-011)

MALAVIKA MOHAN P (2015-06-012)

RESHMA C (2015-06-019)

THOUSEENA T N (2015-06-020)

THESIS

Submitted in partial fulfilment of the requirement for the degree of

BACHELOR OF TECHNOLOGY

IN

FOOD ENGINEERING

Department of Processing and Food Engineering

KELAPPAJI COLLEGE OF AGRICULTURAL ENGINEERING AND TECHNOLOGY

TAVANUR , MALAPPURAM -679573

KERALA, INDIA

2019

DECLARATION

I, hereby declare that this thesis entitled "**Development and quality evaluation of microencapsulated powder of *C. papaya* leaf**." is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Place: Tavanur

Date:

MADHUJA MADHU

(2015-06-011)

MALAVIKA MOHAN P

(2015-06-012)

RESHMA C

(2015-06-019)

THOUSEENA T N

(2015-06-020)

CERTIFICATE

Certified that this thesis entitled ""**Development and quality evaluation of microencapsulated powder of *Carica. papaya* leaf**"." is a record of research work done independently by Ms. Madhuja Madhu(2015-06-011), Ms Malavika Mohan(2015-06-012), Ms Reshma C (2015-06-019), Ms Thouseena T N (2015-06-020) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Tavanur:

Date:

Mrs Sreeja R

Assistant Professor

Kelappaji College of Agricultural Engineering & Technology,Tavanur

ACKNOWLEDGEMENT

Words are no substitute for thoughts and feelings. It is more so when we, the project team, wish to express our sincere thanks to our project guide, **Mrs.Sreeja R,** Asst. Professor, Department of Processing & Food Engineering, KC.A.E.T, Tavanur. Without her constant encouragement, timely advice, patience and readiness to spare and share her treasure of knowledge and experience, this project work would not have progressed to the stage of completion in time. It is with a deep sense of gratitude that we express our heartfelt thanks to her.

With deep sense of gratitude and due respect, we express our heartfelt thanks to **Dr. K.K Sathian**, Dean, K.C.A.E.T, Tavanur for his professional guidance and constructive suggestions offered during this study.

Word would not suffice to express gratitude to all staff members of P&FE, especially **Dr. Santhi Mary Mathew** , Professor and Head of Department of P&FE , **Dr. Prince M V** , Professor, **Er. George Mathew** , Associate professor **, Dr. Rajesh G K ,** Assistant. Professor**, Dr.Riju M C, Er.Nighitha M T** , **Mr.Jabir** for their valuable guidance and constructive criticisms.

We are in short of words to acknowledge the self-less services of **Mr.Radhakrishnan**, **Mrs.Geetha** , **Mrs. Shahma** Technicians without their help this venture would have been impossible for us.

We exploit this opportunity to thank **Library staff, all our batch mates and students of K.C.A.ET** for their sharing and caring which always motivated us throughout the venture.

Words do fail to acknowledge **our parents** support, encouragement and help gone a long way in making this attempt a successful one.

Above all, we bow our head before the **Almighty**, whose grace and blessings have empowered us to complete this toil.

Madhuja Madhu

Malavika Mohan P

Reshma C

Thouseena T N

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF GRAPHS