PHYSICO-CHEMICAL AND NUTRITIONAL ANALYSIS OF SEED AND SEED-BUTTER OF *GARCINIA* SPP.

By

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THESIS

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2023

DECLARATION

I, Urati Mahesh (2020-12-032) hereby declare that this thesis entitled **"Physico-chemical and nutritional analysis of seed and seed-butter of *Garcinia* spp."** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University.

Vellanikkara
17/01/2023

Mahesh.
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CERTIFICATE

This is to certify that this thesis entitled **"Physico-chemical and nutritional analysis of seed and seed-butter of *Garcinia* spp."** is a record of research work done independently by **Mr. Urati Mahesh (2020-12-032)** under my guidance and supervision and that it has not been previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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We, the undersigned members of the advisory committee of **Mr. Urati Mahesh (2020-12-032)**, a candidate for the degree of **Master of Science in Horticulture**, with major field in **Plantation, Spices, Medicinal and Aromatic Crops**, agree that this thesis entitled **"Physico-chemical and nutritional analysis of seed and seed-butter of *Garcinia* spp."** may be submitted by **Mr. Urati Mahesh (2020-12-032)**, in partial fulfillment of the requirement for the degree.

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URATI MAHESH

DEDICATED TO

MY PARENTS

& GUIDE

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ABBREVIATIONS

%	Per cent
/	Per
AOAC	Association of Official Agricultural Chemists
AV	Acid value
°C	Degree Celsius
CBR	Cocoa butter replacer
CCl_4	Carbon tetrachloride
CD	Critical difference
CRD	Completely randomized design
CV	Coefficient of variation
et al.,	et alia (and associates)
etc.	Et cetera
EV	Ester value
FAME	Fatty acid methyl esters
Fig.	Figure
FSSAI	Food Safety and Standards Authority of India
g	Gram
G.	*Garcinia*
GC-FID	Gas chromatography with flame-ionization detection
GC-MS	Gas chromatography-mass spectrometry
GRAPES	General Rshiny Based Analysis Platform Empowered by Statistics
HCA	Hydroxy citric acid
HCl	Hydrochloric acid
i.e.	That is
ICP	Inductively coupled plasma
J	Journal
K	Potassium

KAU	Kerala Agricultural University
KCl	Potassium chloride
Kg	Kilogram
KOH	Potassium hydroxide
M	Metres
meq/kg	Milliequivalent/kg
Mg	Milli gram
ml	Milli liter
Na	Sodium
Na_2CO_3	Sodium carbonate
$Na_2S_2O_3$	Sodium thiosulfate
NaCl	Sodium chloride
$NaNO_2$	Sodium nitrite
NaOH	Sodium hydroxide
nm	Nano meter
OD	Optical Density
SD	Standard deviation
SE (d)	Standard error of difference
Sl. No.	Serial Number
spp.	Species
SPSS	Statistical package for social sciences
SV	Saponification value
tsp	Tea spoon
USA	United States of America
viz.	Namely

INTRODUCTION

1. INTRODUCTION

The genus *Garcinia* belonging to the family Clusiaceae is an underutilized perennial crop found throughout the tropics of Asia and Africa. The genus consists of 200 species (Kar *et al*., 2008). Among these, seven are native to the Western Ghats, six to the Andaman and Nicobar Islands and four to the North Eastern region. Fruiting species of *Garcinia* such as Malabar tamarind or cambodge (*Garcinia gummi-gutta (*L.) Robs.) and kokum (*Garcinia indica* (Thouars) Choisy) are commercially exploited as condiments to flavour a range of food preparations (Khapare *et al*., 2020).

Cambodge fruit rind is acidic and the dried fruit rind is used in the preparation of curries as a souring agent. Kokum fruit rind is of acidic as well as sweet and is mainly used in the preparation of pleasant beverages as well as food preparations.

The *Garcinia* has a significant role in Indian folk medicine and Ayurveda, where it is used to treat flatulence, chronic alcoholism, oedema, dysentery, obesity, diarrhea, bowel dysfunction, *etc*. Apart from these, *Garcinia* species produce valuable non-timber forest products that are lipids, oils, resins and colouring compounds (Khapare *et al.*, 2020). Fruit rind of cambodge and kokum are rich sources of Hydroxy Citric Acid (HCA), an important biologically active plant metabolite used as an anti-obesity and anti-cholesterol drug (Leonhardt and Langhans, 2002; Kim *et al*., 2011). Other medicinal properties of these species are due to secondary metabolites such as alkaloids, glycosides, volatile oils, tannins, flavonoids and phenolic compounds.

Cambodge is grown widely in the homestead of Kerala, whereas kokum trees are cultivated as a traditional homestead crop in coastal and southern interior parts of Karnataka, Goa and Konkan region of Maharashtra. The kokum tree grows naturally in northern Kerala (Rema and Krishnamurthy, 2000). Kottayam and Ernakulam districts of Kerala are known for commercial cultivation and export of cambodge (Tharachand and Avadhani, 2013).

The dried seed of kokum yields edible fat known as kokum butter or *Garcinia* butter which is rich in omega-3 as well as omega-6 fatty acids. Kokum butter is rich in stearic and oleic acids and also contains 75 per cent of mono-oleodisaturated glycerides. Although *Garcinia* butter is edible, it is used to prepare topical cosmetic and medicinal products. The emollient properties of kokum butter find applications in the cosmetic sector (Kirtikar and Basu, 1995). Kokum butter is equivalent to *Vanaspati ghee*. It is often used as a substitute for cocoa butter due to its triglyceride composition. Among different forms of kokum export, oil or butter is important. Countries importing kokum butter from India used in confectionery preparations. After extraction of butter, the unrefined butter from kokum is marketed as egg-shaped lumps; it has a characteristic yellowish colour and greasy nature. It also has a faint but not disagreeable odour. Refined and deodorized fat is white and compares favourably with high-class hydrogenated fat. It is more readily soluble in high temperatures than in the cold.

Similarly, cambodge seed also consists of butter, but it has yet to be commercially characterized. Seeds of both species can be commercially utilized for the extraction of oil or butter. Plenty of seeds after fruit rind collection go as waste, and only a limited quantity of seeds is utilized for seedling production. Preliminary analysis on the composition of *Garcinia* spp. seeds revealed that they deserve to be examined as prospective sources of fat for possible usage as food or feed to fill the gap of oil deprivation (Ajayi *et al*., 2007).

The socio-economic and cultural constraints and the lack of research studies on the extraction and characterization of seed oil or butter limit the utilization of *Garcinia* seeds. Increasing demand for oil for edible purposes or industrial uses in the domestic market can be met easily if strategies and technologies are developed to convert cambodge and kokum seeds into viable value-added products.

The *Garcinia* seeds are a rich source of protein and fat. It is learnt that few native people in the Western Ghats region of Karnataka are collecting butter or oil from cambodge, which is used to prepare household dishes. It is believed that the consumption of butter from kokum seeds would improve digestion and cure dysentery, pain, heart complaints, piles and tumours. However, no scientific evidence on the chemical, nutritional and pharmaceutical properties of *Garcinia* butter available so far.

In this pretext, the present study entitled "Physico-chemical and nutritional analysis of seed and seed-butter of *Garcinia* spp." was undertaken with the specific objectives to probe biochemical and nutritional analysis of seeds as well as extraction, physico-chemical, nutritional analysis and fatty acid profiling of butter, and also organoleptic evaluation of butter and butter based value added products.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Recently, the food industry has given considerable attention to the use of by-products, waste and underutilized agricultural resources. The rise in oil prices necessitates the search for alternative sources of oil, particularly among non-traditional and under-exploited oil seeds. The excellent information available on the proximate composition, mineral content and other properties of more commonly used oils derived from oil seeds. However, limited information is available pertaining to non-conventional ones like *G. indica* and *G. gummi-gutta*.

Previous studies were conducted on propagation, variability, evaluation and value additions of the rind of both cambodge (*Garcinia gummi-gutta* (L.) Robs.) and kokum (*Garcinia indica* (Thouars) Choisy). The present investigation pertains to the characterization of the physico-chemical and nutritional analysis of seed and seed butter of cambodge and kokum, which has been ignored in previous studies. The relevant literature on the study entitled "Physico-chemical and nutritional analysis of seed and seed-butter of *Garcinia* spp.'' is reviewed in this chapter. Wherever adequate literature is unavailable on cambodge and kokum seeds and seed butter, research relevant to other crop seeds and butter are included.

2.1. *Garcinia* species: an overview of utilization

The distribution of different *Garcinia* spp. in the western ghats was presented in Table 2.1. *G. gummi-gutta* is a multipurpose tree seen naturally in evergreen forest of western Ghats and it is cultivated as homestead crops in backyards of Kerala which is used in food preparations to garnish as condiment. Cambodge resin is used as a pigment in miniature water colour and paintings (Singh, 1993). The rind of cambodge is used to impart unique delicate flavour in fish curries which is a common food preparation in Kerala (Muthulakshmi and Sarah, 1999). Fruit rind is an anthelmintic, hydragogue and emetic used in veterinary medicine as a rinse for cattle oral infections. It is also used to polish silver and gold jewellery and as a substitute for acetic acid in the coagulation of rubber latex. The seed oil is used to manufacture medicines, while the gum is used to make varnish (Singh, 1993; Raju and Reni, 2001). Sundried slices of the fruits are used as folk medicine and for culinary purposes in North Eastern India (Parthasarathy *et al*., 2014).

Anju and Rameshkumar (2017) analysed the bioactive compounds in *Garcinia gummi-gutta* rind and they found that bioactive molecules present in the fruit rind as xanthones, benzophenones and organic acids. Fruit rind also constitutes (-) Hydroxy Citric Acid (HCA) ranging from 10 to 30 per cent. It is a well-known hypo-lipidemic agent and an important food supplement for weight management. The species is a rich source of garcinol and isogarcinol.

Kokum fruit has multiple uses in culinary preparations, pharmaceuticals, ayurvedic therapy, nutraceuticals and industries. The fruit extract of kokum has preservative effects in the traditional food preservation in India. Organic acids present in the kokum fruit have a bacteriostatic effect hence, it can be used in a pickling as preservative. It can also be used to lower the pH of the pickling medium (Sreenivasan and Venkatraman, 1959; Lewis *et al*., 1964). Kokum seeds are used for oil extraction and it is used in curries, cosmetics and medicines (Swami *et al*., 2014).

The dried seeds of kokum yield edible fat commonly known as seed butter or kokum butter. This butter is in a solid state at room temperature. Because of the sweet-tangy flavour, the dried rind of kokum fruit is utilized as a substitute for tamarind in cuisine preparations (Jayaprakasha and Sakariah, 2002). Kokum butter is a good source of protein and fat (Abraham *et al*., 2006). The fruits are also used to make wine (Baliga *et al*., 2011). Chate *et al*. (2019) studied the physico-chemical properties of kokum seed flour of full-fat and defatted flour. They have observed that defatted kokum seed flour is of superior quality and contains higher contents of protein, carbohydrates, crude fibre and vitamin B_3 as compared to that of full-fat flour. Kokum juice from the rind is beneficial for curing piles, dysentery, colic problems and diarrhea.

Many small and large-scale sectors related to nutraceutical, food supplementary, cosmetics and beverage industries manufacture significant value-added products from kokum fruits (Swami *et al*., 2014). Several products including kokum syrup, kokum sharbat, kokum agal (kokum juice concentrate), kokum amsul (dry salted rind), kokum beverages and kokum butter are available in the market.

Table 2.1. Distribution of *Garcinia* spp. in the Western Ghats (Shameer *et al*., 2016)

Sl. No.	***Garcinia* species**	**Distribution (Altitude in meter)**	**Area where species found**
1.	*G. gummi-gutta*	India, Sri Lanka (50-900 m)	Throughout the evergreen, semi evergreen forests of the Western Ghats
2.	*G. indica*	Endemic to India, the Western Ghats, North East India (50- 550 m)	Kerala: Thaliparamba, Badi Baduka; Karnataka: Tinai Ghat; Maharashtra: North Kanara, Thungar Hill; Assam: Karbi Anglong Dist.
3.	*G. imberti*	Endemic to South Western Ghats (900-1200 m)	Kerala: Agasthyamala Biosphere Reserve (Trivandrum), Shendaruni (Kollam), Shankily.
4.	*G. wightii*	Endemic to South Western Ghats (250-700 m)	Kerala: Athirappally, Vazhachal, (Thrissur); Paniyeli-poru (Eranakulam)
5.	*G. morella*	Indo-Malayan, Sri Lanka (500- 1100 m)	Kerala: Chenathnair, Kambamala, Kuruva Island (Wayanad); Vellarimala, Thamarassery (Kozhikode); Payampara, Kodakkalthodu (Thrissur); Silent Valley (Palakkad); Pampa (Pathanamthitta); Chemmunjii, Pandimotta, Attayar (Thiruvananthapuram); Karnataka: Horanad Forests; Tamil Nadu: Anaimalai Hills, Kannikketyy, lyyerpadi; Assam: Rani Dawa bang, Pasighat.
6.	*G. pushpangadaniana*	Endemic to the Western Ghats (850-1400 m)	Kerala: Kadalar, Munnar, Pampadumchola (Idukki); Wallakad of Silent Valley (Palakkad); Tamil Nadu: Anaimalai Hills

7.	*G. taibotii*	Endemic to the Western Ghats (100 -500 m)	Kerala: Cheemani, Uduma (Kasaragod); Vazhachal (Thrissur); Vellarimala (Kozhikode); Pandarakayam, Pampa (Pathanamthitta); Rosemala , Pandimotta (Thiruvananthapuram)
8.	*G. rubro-echinata*	Endemic to South Western Ghats (800-1200 m)	Kerala: Chemmunji Hills, Ponmudi (Thiruvananthapuram). Tamil Nadu: Kalakkad Mundanthuarai Tiger Reserve (Thirunelveli)
9.	*G. travancorica*	Endemic to South Western Ghats (950-1500 m)	Kerala: Chemmunjii, Athirumala. (Thiruvananthapuram). Tamil Nadu: Kalakkad Mundanthuarai Tiger Reserve (Thirunelveli)

2.2. Biochemical and nutritional composition of *Garcinia* seeds

Kokum fruit contains 3 to 8 large seeds embedded in the white pulp. The seeds are arranged in a regular pattern similar to orange segments (Nayak *et al*., 2010). Chate *et al*. (2019) studied the physico-chemical properties of kokum (*Garcinia indica)* seed flour with full fat and defatted flour. They noticed that defatted kokum seed flour is of superior quality which contains higher content of protein, carbohydrates, crude fiber and vitamin B_3 as compared to that of full fat flour. Wang *et al.* (2020) isolated gum resin from *Garcinia kola* seeds and it was subjected to high performance liquid chromatography analysis. They observed four biflavanones and derivatives of garcinoic acid in seed gum resin.

2.2.1. Moisture

The moisture level of the seed significantly influences fungal colonization throughout the pre-harvest, post-harvest and storage periods. The seed moisture content is known to affect variations in the chemical makeup of the seeds. The moisture level of the seed during storage is the most potent element that influences the longevity of the seed. Pawar *et al*. (2018) reported the moisture content of kokum seeds as 7.34 per cent. The moisture content of kokum seeds remains stable when stored in polyethylene bag, whereas moisture content varies when the seeds are stored in jute and cloth bags. Chate *et al*. (2019) observed the moisture content of full-fat flour (10.20 %) and defatted flour (8.60 %) in *G. indica.*

Ajayi *et al*. (2007) stated that the moisture content of *Garcinia mangostana* seed as 13.08 ± 1.99 per cent. Sonawane *et al*. (2014) studied the physical characteristics of kokum seeds and reported a moisture range of 7.35 to 25.79 per cent on a dry basis. The bulk density grew, the actual density fell, the interconnected porosity reduced, the repose angle increased and the terminal velocity increased as the moisture content climbed from 7.35 to 25.79 per cent dry basis. Manohar *et al*. (2014) reported that the moisture content of *Garcinia xanthochymus* seed is 11.96 ± 0.52 per cent stating that it may be stored for an extended amount of time without deteriorating.

2.2.2. Starch

Starch is an essential source of nutrition in a regular diet. Starch is generated in growing seeds, where it serves as an energy source for seedling establishment. Noor *et al*. (2016) analysed the starch content in seeds of four species of *Garcinia* such as *G. mangostana, G. hombroniana, G. prainiana* and *G. atroviridis.* They have recorded a maximum amount of starch in *G. mangostana,* whereas a minimum amount of starch in *G. hombroniana, G. prainiana* and *G. atroviridis.* The recalcitrant seeds may use starch in lipid production, as lipids are considered as storage reserves. Murmu *et al*. (2016) reported that the starch content in raw fruit of *Garcinia xanthochymu*s as 9.06 per cent.

2.2.3. Total carbohydrates

Parthasarathy and Nandakishore (2014) reported that carbohydrates are the major metabolites found in *Garcinia* species. The total carbohydrates content varied in fruits of different *Garcinia* species such as *G. gummi-gutta* (6.46 g per 100 g), *G. indica* (5.67 g per 100 g), *G. mangostana* (15.12 g per 100 g), *G. xanthochymus* (3.75 g per 100 g), *G. subelliptica* (4.38 g per 100 g), *G. kydia* (8.25 g per 100 g), *G. lanceaefolia* (5.32 g per 100 g) and *G. pedunculata* (7.21 g per 100 g). Total carbohydrate content was recorded higher in fruits of *G. mangostana*. Chate *et al*. (2019) reported that the carbohydrate content of kokum full fat flour and defatted flour was 29.70 per cent and 58.50 per cent, respectively. Ikeda *et al*. (2021) analysed the carbohydrate content of different growing stages of seeds of *Garcinia humilus*. The growing stages such as unripe, mid-ripe and full-ripe seeds, varied for the carbohydrate content 54.40 g per 100 g, 40.00 g per 100 g and 42.00 g per 100 g, respectively. Melo *et al*. (2022) reported the carbohydrate content of unripe seed flour and ripe seed flour of *Garcinia brasiliensis*. Carbohydrate content is higher in the case of unripe seed flour (61.98 g per 100 g) compared to ripe seed flour (55.14 g per 100 g).

2.2.4. Proteins

Parthasarathy and Nandakishore (2014) reported the protein content in the fruits of various *Garcinia* species such as *G. gummi-gutta* (3.25 g per 100 g), *G. indica* (4.78 g per 100 g), *G. mangostana* (1.82 g per 100 g), *G. xanthochymus* (4.01 g per 100 g), *G. subelliptica* (3.76 g per 100 g), *G. kydia* (4.33 g per 100 g), *G. lanceaefolia* (3.45 g per 100 g) and *G. pedunculata* (4.93 g per 100 g). Protein content was recorded higher in *G. pedunculata* followed by *G. indica*. Next to carbohydrates, proteins are the major metabolites found in *Garcinia* species. Ikeda *et al*. (2021) reported the protein content of *Garcinia humilus* at different developing stages of seeds. Protein content varied in unripe (2.4 g per 100 g), mid ripe (3.0 g per 100 g) and full ripe (3.2 g per 100 g) seeds.

Noor *et al*. (2016) reported that the proteins were available in small amount in seeds of *Garcinia* species such as *G. mangostana, G. hombroniana, G. prainiana* and *G. atroviridis*. Chate *et al*. (2019) reported that the protein content of kokum full-fat flour and defatted flour was 9.10 and 15.00 per cent, respectively. De Melo *et al*. (2022) reported the protein content in bacupari (*Garcinia brasiliensis)* of unripe seed flour (5.07 g per 100 g), ripe seed flour (5.20 g per 100 g), unripe peel flour (6.61 g per 100 g) and ripe peel flour (5.48 g per 100 g). The protein content of the peel flour was higher than seed flour in both ripe and unripe fruits.

2.2.5. Total fat

Lipids, sometimes known as fats and it is hydrophobic hydrocarbon molecules. Fats are a kind of energy storage found in plants, particularly in seeds. Fats are the second most abundant source of energy for living cells. Parthasarathy *et al*. (2014) reported the total fat content of *Garcinia gummi-gutta* (46.5 %), which is higher when compared to *Garcinia indica* (29.3 %), *Garcinia* xanthochymus (25.70 %) and *Garcinia mangostana* (24.20 %). Histochemical investigation revealed that the seeds of *Garcinia* species such as *G. mangostana, G. hombroniana, G. prainiana* and *G. atroviridis* contain a high lipid concentration (Noor *et al*., 2016). Nagavekar *et al*. (2019) recorded the fat content of *Garcinia indica* seeds through hot water skimming and the Soxhlet method of extraction. They found that the highest fat is recorded through the Soxhlet method of extraction (42.80 %) than that of the hot water skimming method (25.30 %). Raveena (2019) observed the fat content in sixteen accessions of kokum seeds; it ranged from 25.65 to 35.69 per cent.

2.2.6. Dietary fibre

Dietary fibre is a category of plant components that cannot be fully digested in our intestines. Dietary fibre adds bulk to your diet and aids digestion, prevents constipation and helps control of body weight. Tosh and Yada (2010) reported the dietary fibre of various pulses like beans (23-32 g per 100 g), chickpeas (18-22 g per 100 g), lentils (18-20 g per 100 g) and peas (14-26 g per 100 g). The dietary fibre was recorded higher in pulses due to thicker seed coat or hull promoting variety of physiological effects that are beneficial to human health. Ikeda *et al*. (2021) reported the dietary fibre of achachairu (*Garcinia humilus)* at different seed-growing stages. Dietary fibre is almost similar in the case of unripe (28.30 g per 100 g), mid ripe (28.30 g per 100 g) and full-ripe (28.20 g per 100 g) seeds. Similar amount of dietary fibre was observed in unripe, mid ripe and full ripe seeds.

2.2.7. Total ash or Total minerals

The ash content represents the incombustible component that remains after a plant sample is completely burned. Ebana *et al*. (2017) reported that the ash content of *Garcinia kola* seeds of 3.50 per cent. Seeds of *Garcinia kola* have the mineral composition of Na (10.10 mg per 100 g), K (730.00 mg per 100 g), Ca (200.40 mg per 100 g), Mg (170.50 mg per 100 g) and Fe (4.20 mg per 100 g). Chate *et al*. (2019) observed the ash content of full-fat flour (2.90 %) and defatted flour (4.80 %) in *G. indica.* Ash content of defatted flour was higher than full-fat flour making it as a supplement in various food systems. Seed flour of kokum varied with a mineral composition such as sodium (34.80 mg per 100 g), calcium (24.10 mg per 100 g), iron

(8.78 mg per 100 g) and zinc (0.993 mg per 100 g). Ikeda *et al*. (2021) reported the ash content of achachairu (*Garcinia humilus)* at different developing stages of seeds. Ash content varied in unripe (1.10 g per 100 g), mid-ripe (1.70 g per 100 g) and full ripe (2.20 g per 100 g) seeds. The high ash content of seed flour indicates the presence of considerable amounts of phosphorous (52.86 mg per 100 g) and potassium (224.56 mg per 100 g) along with minor amounts of minerals such as manganese, calcium, magnesium, zinc, copper and iron.

According to Ajayi *et al*. (2007), the ash content of *Garcinia mangostana* seeds of 1.99 g per 100 g, which is higher than the values reported for coconut, kola nut and melon but lower than those determined for castor, groundnut and oil bean seeds. Manohar *et al*. (2014) reported that the ash content of *Garcinia xanthochymus* seeds of 2.35 g per 100 g. De Melo *et al*. (2022) reported the ash content of bacupari (*Garcinia brasiliensis)* in unripe seed flour, ripe seed flour, unripe peel flour and ripe peel flour as 1.61, 2.09, 4.37 and 4.24 g per 100 g, respectively. The ash content of seed flour is less than that of peel flour indicating the presence of low mineral content in seed flour compared to peel flour.

Parthasarathy and Nandakishore (2014) reported the mineral composition *viz.* calcium, iron, potassium and sodium in fruits of different *Garcinia* species such as *G. indica* (13.21, 12.06, 44.5 and 1.55 mg per 100 g, respectively), *G. gummi-gutta* (12.67, 9.00, 26.6 and 2.88 mg per 100 g, respectively), *G. mangostana* (5.82, 9.02, 78.3 and 2.58 mg per 100 g, respectively), *G. xanthochymus* (13.07, 10.82, 28.4 and 2.06 mg per 100 g, respectively), *G. subelliptica* (12.33, 9.0, 43.3 and 1.52 mg per 100 g, respectively), *G. kydia* (12.54, 10.00, 38.7 and 2.54 mg per 100 g, respectively), *G. lanceaefolia* (12.54, 9.00, 52.3 and 1.35 mg per 100 g, respectively) and *G. pedunculata* (13.21, 10.12, 27.3 and 2.48, respectively). Potassium and calcium were found to be pre-dominant minerals in fruit of *Garcinia*.

Raysad (2016) analysed the mineral composition of butter extracted from thirty-two accessions of *Garcinia gummi-gutta*. Significant variation in mineral content *viz.* Ca (105.44 to 212.25 mg per 100 g), Na (41.00 to 98 mg per 100 g), K (115.80 to 227.10 mg per 100 g), P (20.40 to 52.40 mg per 100 g) and Mg (10.00 to 12.87 mg per 100 g) was recorded in accessions of cambodge. Raveena (2019) studied the nutritional content of butter extracted from sixteen accessions of *Garcinia indica*.

Variation in mineral composition *viz*. Ca (4.56 to 7.45 mg per 100 g), Na (0.23 to 1.92 mg per 100 g), K (25.00 to 33.05 mg per 100 g), P (2.09 to 4.85 mg per 100 g) and Mg (17.20 to 27.98 mg per 100 g) was observed in accessions of kokum. According to Joseph *et al*. (2017), good amount of minerals *viz.* sodium (840.00 mg per 100 g), potassium (1036.00 mg per 100 g), calcium (420 mg per 100 g) and iron (34.00 mg per 100 g) were recorded in seeds of African mangosteen.

2.3. Extraction, physico-chemical and nutritional analysis and profiling of butter

Sonawane *et al*. (2014) described multiple applications of kokum butter. The colour of the kokum butter is light grey or yellowish. It is used in the preparation of vaseline to treat skin cracks. It also finds use in treating dysentery. Since it has pine tallow-like qualities, since it has pine tallow-like qualities, it can be used for pharmaceutical preparations, suppositories, cosmetic preparations and ointments. Due to the nourishing and emollient properties of kokum butter, it has applications in cosmetics. The demulcent and astringent actions of butter find their importance in medicinal applications. Apart from these usages, it is utilized to make soap and candles as well as for confectionery.

Praneetha and Balamohan (2014) documented that the butter extracted from kokum seeds is primarily used for household consumption and to prepare dishes such as *dosa* (pan cake), *payasa* (a sweet dish), *kadubu* (steamed rice dish) and other food products. In the Konkan region of Maharashtra, kokum butter is used to smear hot rotis. Also, residents consume kokum butter daily due to its pharmacological benefits like cardio tonic and anthelmintic properties; to cure tumors, pain, dysentery and heart complaints and treat piles. The kokum butter is reported to be the most effective therapy for ailments such as worm infection when applied to fissures and ulcerations of the lips, hands *etc*.

2.3.1. Extraction of butter

Supercritical Fluid Extraction (SFE) using liquid CO_2 extracts kokum fat from the seeds of *Garcinia indica*. Compared to Soxhlet extraction with petroleum ether (42.8 % fat content), supercritical fluid extraction at 350 bar, 60 °C for 360 min yielded a maximum fat recovery of 90.24 per cent. Hot water skimming of fat extraction in kokum seeds yields up to 25.3 per cent. Soxhlet extraction of butter was found to be the most efficient method by scanning electron microscope (SEM) analysis of the spent biomass, followed by supercritical Fluid Extraction (SFE) and hot water skimming method (Nagavekar *et al*., 2019).

According to Vidhate and Singhal (2013), edible fat from kokum can be recovered at a low cost by crumbling the seed kernels, boiling them in water and scooping off fat from the top of the container. In this method, by-products of poor quality and recovery of about 25 to 31 per cent are the most significant constraint of kokum fat extraction. Fat can also be produced using solvent extraction with hexane. However, this process is only cost-effective when followed on a big scale. TPP (Three-Phase Partitioning) was an improved methodology using ammonium sulphate concentration, slurry to t-butanol ratio and slurry pH to obtain maximum fat recovery of 95 per cent (w/w) in about 2 hours, compared to soxhlet extraction of butter using petroleum ether (49 %). The method is cost-effective as well as environmentally friendly, which has the potential to convert agro-processing waste into a commercially viable product.

Bhande and Giri (2017) investigated kokum oil recovery considering two parameters like solvents (petroleum ether and chloroform) and particle size of seed powder (1000 microns and 1200 microns). With chloroform and particle size of 1000 microns, oil recovery was 83.63 per cent when compared to petroleum ether and the same particle size oil yield was 78.78 per cent. They have concluded that a tiny sample particle size (1000 microns) can boost oil recovery and solvents have minimal effect on oil recovery independent of particle size. In another study by Bhande *et al*. (2017) analysed oil recovery of kokum by employing two parameters like solvents (hexane and ethanol) and particle size (850 microns and 1200 microns). Hexane with a particle size of 850 microns could achieve a high oil recovery of 95.51 per cent. It was concluded that the sample's particle size is reduced (850 microns) and the oil content can be increased.

2.3.2. Physico-chemical analysis

2.3.2.1. Physical state at room temperature

The term "butter" or "oil" refers to fats, which are a subset of lipids. Oil is commonly referred to as fats that are liquid at room temperature; butter is also referred to as fats that are solid at ambient temperature (Richard, 2010). Parthasarathy *et al*. (2014) reported the physical condition of butter extracted from four *Garcinia* species such as *Garcinia gummi-gutta*, *Garcinia indica*, *Garcinia xanthochymus* and *Garcinia mangostana.* Butter from this species is solid at room temperature. Raysad (2016) observed that cambodge butter is solid at room temperature. Raveena (2019) stated that the physical state of kokum butter is solid at room temperature.

2.3.2.2. Moisture content

The moisture level of butter during storage is the most potent element that influences the quality and longevity of the butter. Chukwu and Adgidzi (2008) reported that the moisture content of shea butter is 1.37 per cent. O-Ebongue *et al*. (2013) recorded moisture content of crude palm oil as 0.20 per cent and stated that the high moisture content accelerates butter's spoilage and decreases food quality. In another study, Ramachandran (2014) observed that the moisture content of kokum butter is 0.50 per cent

2.3.2.3. Total fat or Oil content

According to FSSAI (2017), the fat content reported in milk butter was 80-83 per cent.

2.3.2.4. Refractive index

The refractive index indicates the possible chances of rancidity development in oil. The higher the refractive index higher is the chances of spoilage due to oxidation. The refractive index is an important optical parameter for analysing the light rays traversing through a material medium. Ajayi *et al*. (2007) reported that the refractive index of *Garcinia mangostana* seed oil was 1.482. Manohar *et al*. (2014) recorded the refractive index of *Garcinia xanthochymus* seed oil as 1.488. In another study by Chukwu and Adgidzi (2008), the refractive index of shea butter was 1.452 per cent at 40 °C.

2.3.2.5. Melting point and Boiling point

The constant temperature at which a substance changes from a solid to a liquid state is called the melting point. The boiling point is the constant temperature at which a substance changes from a liquid to a gaseous state. Nag *et al*. (1995) reported the boiling point of *Putranjiva roxburghii* (255-261 ℃). According to Liendo *et al*. (1997), the melting point of cocoa butter ranged from 35 to 36 ℃. Parthasarathy *et al*. (2014) reported that the melting point of *Garcinia gummi-gutta* (39.40 ℃) which was followed by *Garcinia indica* (40.30 ℃), *Garcinia xanthochymus* (38.20 ℃) and *Garcinia mangostana* (37.90 ℃). They stated that *Garcinia* seed butter has a high melting point (about 40 ℃), which can be substituted with cocoa butter to improve chocolate's hardness and heat resistance behaviour. Raveena (2019) reported that butter's melting point (℃) in sixteen accessions of kokum ranged from 35 to 44 ℃. Raysad (2016) recorded the melting point of butter in thirty-two accessions of the cambodge which ranged from 36 to 42 ℃.

2.3.2.6. Solubility

Solubility is the property of a solid, liquid or gaseous chemical substance called a solute that dissolves in a solid, liquid or gaseous solvent to form a homogeneous solution of the solute in the solvent. Vidhate and Singhal (2013) reported that kokum butter is more soluble in t-butanol compared to ethanol and iso-propanol.

2.3.2.7. pH

Jagtap *et al*. (2015) opinioned that kokum fruits are very acidic in nature with a pH range of 1.5 to 2.5. Adewumi *et al*. (2017) reported the pH of milk (7.01), butter (6.01) and garlic butter (5.85). It was observed that pH reduces with an increased lactic acid content which causes milk to clog in butter during churning.

2.3.2.8. Acid value

The acid value represents an oil or fat's freshness and storage quality. It is the measure of susceptibility and extent of decomposition. Parthasarathy *et al*. (2014) identified the acid value of *Garcinia* spp.; it varied from 3.7 to 4.9 mg NaOH per g.

The acid value of *G. gummi-gutta (*3.70 mg NaOH per g), *G. indica (*4.90 mg NaOH per g), *G. xanthochymus* (4.80 mg NaOH per g) and *G. mangostana* (4.50 mg NaOH per g); it shows that butter was suitable for the consumption in daily diet.

Choppa *et al*. (2015) reported that the acid value of Malabar tamarind seed oil (5.04 mg KOH per g) is high when compared to sunflower oil (3.09 mg KOH per g) and it was lower in olive oil (6.60 mg KOH per g). Raysad (2016) reported the acid value of *G.* gummi-gutta for different accessions ranging from 3.09 to 4.49 mg KOH per g. It was observed that the highest acid value of 4.49 mg KOH per g in IC244094-1, IC244077-1-3 and IC244084-2, whereas the lowest acid value of 3.09 mg KOH per g was recorded in IC244077-12. The mean acid value observed among the accessions of the cambodge was 3.80 mg KOH per g. Raveena (2019) studied the acid value in sixteen accessions of *G. indica.* It ranged from 2.60 (IC342327-1, IC342303-2) to 3.40 (IC1336687-3) mg KOH per g. The mean acid value observed among the kokum accessions studied was 2.95 mg KOH per g.

2.3.2.9. Saponification value

The saponification value is a measure of the average molecular weight or chain length of all the fatty acids present in the sample of triglycerides. The higher the saponification value, the lower the fatty acids average length and the lower the mean molecular weight of triglycerides. Practically, fats or oils with high saponification value are more suitable for soap making. Saponification value of *Garcinia* spp. *viz. G. gummi-gutta (*187.90 mg KOH per g), *G. indica* (200.2 mg KOH per g), *G. xanthochymus* (190.30 mg KOH per g) and *G. mangostana (*140.50 mg KOH per g) oil. The saponification value of olive oil was 187 to 196 mg KOH per g; sunflower oil ranged from 188 to 194 mg KOH per g; ground nut oil was ranged from 188 to195 mg KOH per g; mustard oil varied from 169 to176 mg KOH per g and sesame oil ranged from 188 to 195 mg KOH per g. In comparison, it is very high in coconut oil (251-263 mg KOH per g) and ghee (220 mg KOH per g) as reported by Parthasarathy *et al*. (2014).

Raysad (2016) reported that the saponification value of *G. gummi-gutta* accessions ranged from 171.81 to 189.34 mg KOH per g. The maximum saponification value was recorded in IC244101-2 and IC244084-2 (189.34 mg KOH per g). In comparison, the minimum saponification value was recorded in IC244094-1 (171.81 mg KOH per g). Raveena (2019) reported that the saponification value in sixteen accessions of *G. indica* ranged from 171.10 mg KOH per g (IC 136687-2) to 194.94 mg KOH per g (IC 552526-2). The mean saponification value among the accessions of kokum was 183.98 mg KOH per g. The mean saponification value observed among accessions of the cambodge was 181.62 mg KOH per g. According to Ramachandran (2014), the saponification value of kokum butter varied from 187.00 to 193.00 mg KOH per g of oil.

2.3.2.10. Iodine value

The iodine value is a measure of the unsaturated qualities of the fat. An iodine value between 25 and 50 is considered as ideal. The iodine value obliges in determining the tendency of fat to become rancid. The iodine value is meagre in coconut oil (7.5- 10.5); hence it tends to get rancid quickly. The iodine value of *Garcinia* spp. oil was studied, such as *G. gummi-gutta* (50.20 g per 100 g), *G. indica* (39.40 g per 100 g), *G. xanthochymus* (37.40 g per 100 g) and *G. mangostana* (51.80 g per 100 g). The iodine value of olive oil is 79-90 g per 100 g, for sunflower it is 125-140 g per 100 g, for ground nut it is 84-100 g per 100 g, for mustard oil it is 98-110 g per 100 g, for sesame oil it is 103-116 g per 100 g and for palm oil it is 4-22 g per 100 g oil (Parthasarathy *et al*., 2014).

Raysad (2016) observed the iodine value of *G. gummi-gutta* accessions, that was ranged from 48.22 to 76.14 g per 100 g oil. It was observed that the highest iodine value of 76.14 g per 100 g in IC2447743, whereas the lowest iodine value of 48.22 g per 100 g was recorded in IC244094-1. The mean iodine value observed among accessions of cambodge was 60 g per 100 g. Raveena (2019) reported that there is less variation in the iodine value of kokum in 16 accessions. The mean iodine value observed among the accessions of kokum butter was 38.63 g per 100 g.

Kalse *et al*. (2021) studied the physico-chemical properties of kokum and cocoa butter and they have reported that the iodine value of kokum butter (32-40 g per 100 g) and cocoa butter (32-35 g per 100 g) were comparable.

2.3.2.11. Peroxide value

The peroxide value of fats denotes the degree of primary oxidation, thus, the likelihood of rancidity. A lower peroxide value indicates good oil composition and good storage condition.

The peroxide value of the oil from *G. mangostana* seeds was 3.27 ± 0.12 mg per g, which was suitable for the edible purpose (Ajayi *et al*., 2007). Manohar *et al*. (2014) reported that the peroxide value of *Garcinia xanthochymus* seed oil was 13.9 ± 1.46 (mg per g oil) and stated that it might be stored for an extended period without deteriorating. Findik and Andic (2017) studied the peroxide value of butter and butter oil made from the same raw material. They have observed that the peroxide levels were more significant in butter oil than in butter samples. Kalse *et al*. (2021) studied the physico-chemical properties of kokum and cocoa butter. They have reported that the peroxide value of kokum butter was 5 meq per kg and cocoa butter was 1.00-1.10 meq per kg.

2.3.2.12. Ester value

The highest ester value reveals the presence of a high amount of ester and low molecular weight fatty acid content. Songkro *et al*. (2010) recorded that the ester value of virgin coconut oil was 254.6 mg KOH per g. Hesham *et al*. (2015) observed the ester value of different lipids such as olive oil (142.5 mg KOH per g), marula traditional oil (308.16 mg KOH per g), melon oil (142.4 mg KOH per g), sunflower oil (124 mg KOH per g), marula cooking oil (126.44 mg KOH per g) and ximena oil (106.93 mg KOH per g). Mohammed *et al*. (2017) reported that the ester value of *Garcinia indica* was 157.9 mg KOH per g.

2.3.3. Nutritional analysis of butter

The residue of an oil's organic component left over after it is burned off in the air is known as its ash content. Inorganic components are present in the form of oxides in the ash. Galvao *et al*. (1976) reported the mineral composition of peanut butter such as Ca (46.70 mg per 100 g), K (682.00 mg per 100 g), Na (388.00 mg per 100 g), Cl (581.00 mg per 100 g), Mg (148.00 mg per 100 g), Fe (1.62 mg per 100 g) and Zn (2.91 mg per 100 g). Chukwu and Adgidzi (2008) reported the ash content of shea butter (1.26 %) and groundnut oil (1.8-3.1 %) indicating the presence of minerals in butter and oil. According to FSSAI (2017), the nutritional composition of milk butter was reported as calcium (18.00 mg per 100 g), sodium (9.00 mg per 100 g) and potassium (27.00 mg per 100 g).

2.4. Fatty acid profiling of butter

Niveditha (2013) reported the fatty acid composition of the four morpho types of kokum. A total of seven fatty acids were detected using Gas Chromatography (GC) such as capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid and linoleic acid. Stearic acid and oleic acid are the dominant fatty acids observed in the rinds of kokum. Orange morpho types showed the highest stearic acid (60.88%), whereas red morpho types showed the highest oleic acid (39.17%) compared to the yellow and green morpho types.

Parthasarathy *et al*. (2014) reported the fatty acid content of different *Garcinia* species. The fatty acids such as stearic acid, oleic acid, palmitic acid and linoleic acid varied among the *Garcinia* species *viz. G. indica* (49.33 %, 34.42 %, 3.25 %, 5.25 %, respectively), *G. gummi-gutta* (30.61 %, 26.23 %, 6.31 %, 11.38 %, respectively), *G. mangostana* (2.31 %, 34.02 %, 47.20 %, 1.32 %, respectively) *and G. xanthochymus* (44.53 %, 35.33 %, 3.05 %, 4.82 %, respectively).

Ramachandran (2014) reported the occurrence of free fatty acids in kokum butter ranged upto 7.2 per cent. The composition of fatty acids such as stearic acid (50-65 %), palmitic acid (2-8 %), linoleic acid (0-8 %) and oleic acid (30-44 %) makes the butter appeal in daily usage.

According to Jagtap *et al*. (2015), seed butter of kokum consists of abundant glycerides of stearic acid (55%), oleic acid (40%), hydroxyl capric acid (10%), palmitic acid (3%), linoleic acid (1.5%) and myristic acid (0.5%). About 7.2 per cent of free fatty acids were recorded in kokum butter which makes the butter as excellent emollient used by cosmetic industry.

GC-MS analysis of kokum butter recorded fatty acids such as stearic acid (65.8%), oleic acid (32.5%) and palmitic acid (1.5%). These fatty acids often make the butter to substitute cocoa butter in chocolate preparations (Nagavekar *et al*., 2019).

2.5. Organoleptic evaluation butter

A highly acceptable burfi has a moderately sweet taste, a sightly greasy body with a smooth texture, good breaking strength and very fine graininess (Chetana *et al*., 2010). Tiwari *et al*. (2011) reported the sensory evaluation of burfi prepared using *margarine*, sugar, khoa, ghee, glucose powder and butter. The colour, graininess, texture was light in burfi prepared with butter, margarine (palm oil and coconut oil (80:20) and ghee, respectively. Burfi prepared with margarine and ghee are highly accepted for overall quality due to textural properties.

Kalse *et al*. (2021) investigated the physico-chemical properties of kokum and cocoa butter and reported that many of the components were comparable among the samples. The findings demonstrated that kokum butter could be utilized as an alternative to cocoa butter, thereby lowering the cost of chocolate production, which could be renumerated to kokum sectors.

Edible fat is extracted from the seed kernels of Malabar tamarind in the state of Karnataka and districts of Uttara Kannada and Dakshina Kannada; this is being utilized in the preparation of delicacies by traditional households on special occasions. *Holige*, a sweet dish made with bengal gram powder and *pancha kajaye*, a sweet dish made with powdered bengal gram, cardamom, sesame and copra are both prepared using edible fat (Abraham *et al*., 2006).

Increased demand and shortage of cocoa butter as well as poor quality of individual harvests, economic benefits and some technological advantages have prompted the development of a substitute known as Cocoa Butter Replacer (CBR). Due to a close association with several physicochemical qualities, kokum butter is viable as a substitute for cocoa butter (Naik and Kumar, 2014).

Kokum fat and Phulwara butter fraction were used as cocoa butter extenders in chocolate and confectionery preparations. Phulwara butter fraction was mixed with Kokum fat in various amounts to produce a wide range of hard butter with varying melting qualities. Blends with more significant levels of kokum fat were tougher in hotter climates, making them ideal for use in chocolate and confectionery (Reddy and Prabhakar, 1994).

Cocoa butter is the perfect fat for chocolate which softens on heat and should not be used in hot areas. Chocolate firmness can be improved by adding kokum fat. Plastic viscosity or yield stress of milk or dark chocolate was not significantly impacted by adding kokum fat up to five per cent to the product's weight. Kokum fat was added to increase the hardness of dark and milk chocolates (Maheshwari and Reddy, 2005).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

Cambodge and kokum are perennial tall growing dioecious trees with alternate bearing nature. The fruiting seasons of cambodge and kokum are distinct. The peak fruiting period in cambodge is from June to July and the kokum fruiting season is from January to April. Collection of ripe fruits was done during the peak period of fruiting season from the selected accessions of cambodge and kokum.

3.1. Collection of seed materials

Different accessions of cambodge and kokum were selected for collection of seeds. Each accession consists of single tree. Seeds collected from different accessions of cambodge and kokum are depicted in Table 3.1.

Table 3.1. Cambodge and kokum accessions used in the study

***Garcinia* spp.**	**Accessions**	**Source of collection**
Cambodge	ACC.FSC.C-1	*Garcinia* block, Dept. of Fruit Science, COA, Vellanikkara.
	ACC.PSMA.1	Plantation and Spice farm, Dept. of Plantation, Spices, Medicinal and Aromatic Crops, COA, Vellanikkara
	IC244101-2	ICAR-NBPGR, Regional Station, Vellanikkara, Thrissur
Kokum	ACC.FSC.1	College orchard, Dept. of Fruit Science, COA, Vellanikkara.
	IC342296-1	ICAR-NBPGR, Regional Station, Vellanikkara, Thrissur
	IC342302-2	ICAR-NBPGR, Regional Station, Vellanikkara, Thrissur

3.1.1. Extraction of seed kernels from fruits

Ripen fruits were collected from cambodge and kokum trees during peak period of harvesting and split into two halves. Seeds were collected along with the pulp. Seeds were washed vigorously to remove the adhering pulp, which makes the seeds dry faster. The drying of seeds was done using hot air oven maintained at temperature of 50-60 °C for 4 to 5 days. In case of kokum, sun drying was tried as the harvesting coincides with the summer season. Seeds were dried until the seed coat become brittle and the kernels were collected after removing the seed coat. Collected kernels were utilized in the study.

3.1.2. Preparation of cambodge and kokum seed powder

Seed powder of cambodge and kokum was prepared using dried seeds or kernels. The steps followed for the preparation of cambodge and kokum seed powder is depicted in Fig. 3.1.

3.2. Biochemical and nutritional analysis of cambodge and kokum seeds

Cambodge and kokum seed powder was examined for biochemical and nutritional parameters using standard operating procedures.

3.2.1. Moisture (%)

The moisture content of seed powder was determined as per the method suggested by Association of Official Agricultural Chemists (AOAC,1980). Moisture content was evaluated by weighing approximately 5 g of seed powder into pre-weighed petri dishes and placing them in a 105°C oven for 12 hours. After cooling in a desiccator, the sample was weighed. This process was done until a steady weight was attained.

$$\text{Moisture content (\%)} = \frac{(B-A) - (C-A)}{(B-A)} \times 100$$

A= Weight of dry scale pan (g)

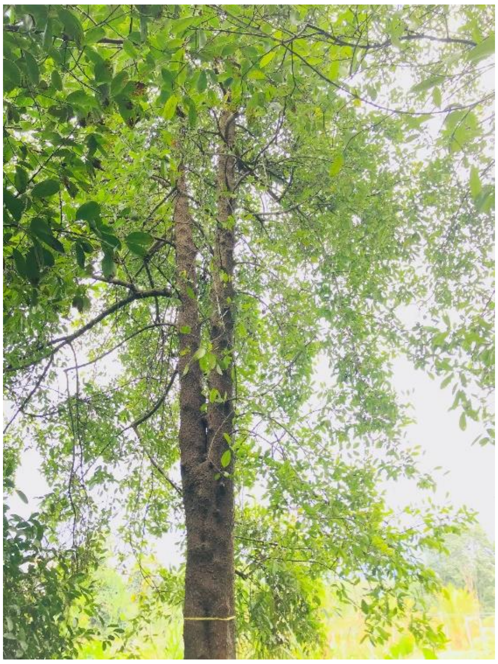
B= Weight of scale pan + seed powder (g)

C= Weight of the scale pan + dried seed powder (g)

IC244101-2 **ACC.PSMA.1**

ACC.FSC.C-1

Plate 3.1. Cambodge accessions used in the study



IC342296-1

IC342302-1

ACC.FSC.1

Plate 3.2. Kokum accessions used in the study

Plate 3.3. Collection of cambodge fruits during peak fruiting period

Collection of cambodge and kokum fruits

↓

Cutting of cambodge and kokum fruits

↓

Separation of seeds from rind and pulp

↓

Cleaning the adhering pulp of seeds

↓

Sun drying or artificial drying of seeds

↓

Storage of seeds for processing

↓

Shelling of seeds by seed dehuller or twisting the seeds by manual method

↓

Winnowing to separate kernels and shell

↓

Storage of kernels for processing

↓

Slight roasting of kernels to remove impurities and make fats loose

↓

Grinding of roasted kernels in a mixer

↓

Packaging of cambodge and kokum seed powder in polythene bags

↓

Stored in a moisture free container or used in further study

Fig. 3.1. Flow chart for the preparation of cambodge and kokum seed powder

3.2.2. Starch (g per 100 g)

The amount of starch in seed powder was quantified calorimetrically at 630 nm using the standard protocol reported by Sadasivam and Manickam (1997).

The weighing of the seed powder samples of 0.5g. The samples were mixed with 80 per cent ethanol to eliminate the sugars. The residue was rinsed several times to complete elimination of the sugars. The residues were dried before being extracted in cold water for 20 minutes; added 5 ml of water and 6.5 ml of 52% perchloric acid. Centrifuged the sample to collect the supernatant. After that, the material was reextracted with new perchloric acid. The supernatant was taken from the sample and diluted to 100 ml. Pipette off 0.2 ml supernatant and add water to make up to 1 ml. Then the anthrone reagent was added (4 ml) and the solution was heated for 8 minutes, cooled and measured at 630 nm.

3.2.3. Total carbohydrates (g per 100 g)

The total carbohydrates content of cambodge and kokum seed powder was estimated using Anthrone method (Sadasivam and Manickam, 1997). A 100 mg dried sample of seed powder was hydrolysed for 3 hours in a water bath with 5 ml of 2.5N HCl and cooled to room temperature. Sodium carbonate was used to neutralise the residue until the effervescence stopped. The volume was increased to 100 ml before centrifugation. An aliquot of 0.2 ml of supernatant was pipetted out and increased to 1 ml before adding 4 ml of anthrone reagent. After heating for 8 minutes in a boiling water bath, followed by cooling rapidly, the intensity of green to dark colour was measured at 630 nm (OD). Using standard glucose solution, a standard graph was prepared by applying repeated dilutions. The amount of total carbohydrates in the sample was determined using the standard graph and represented in grams per 100 g sample.

3.2.4. Protein (g per 100 g)

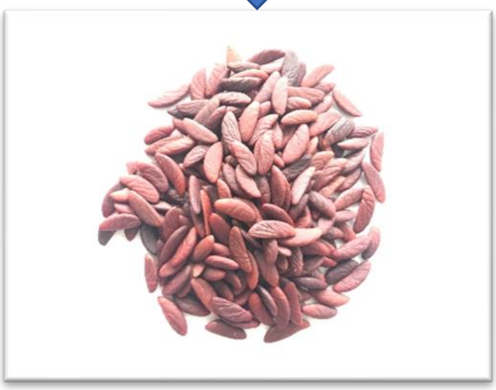
The protein content of cambodge and kokum seed powder was estimated using Lowry's method (Sadasivam and Manickam, 1997). A 500mg sample was extracted using 5 to 10 ml of buffer (Tris buffer GR - tris hydroxymethyl amino methane) and centrifuged. In a test tube, an aliquot of 0.1 ml of supernatant was placed and 5 ml of

Ripen cambodge fruits

Cambodge seeds with pulp

Removal of seed mucilage

Seeds dried in hot air oven



Nibbed kernels

Kernels packed in polythene bags

Plate 3.4. Steps followed for extraction of cambodge seed kernels

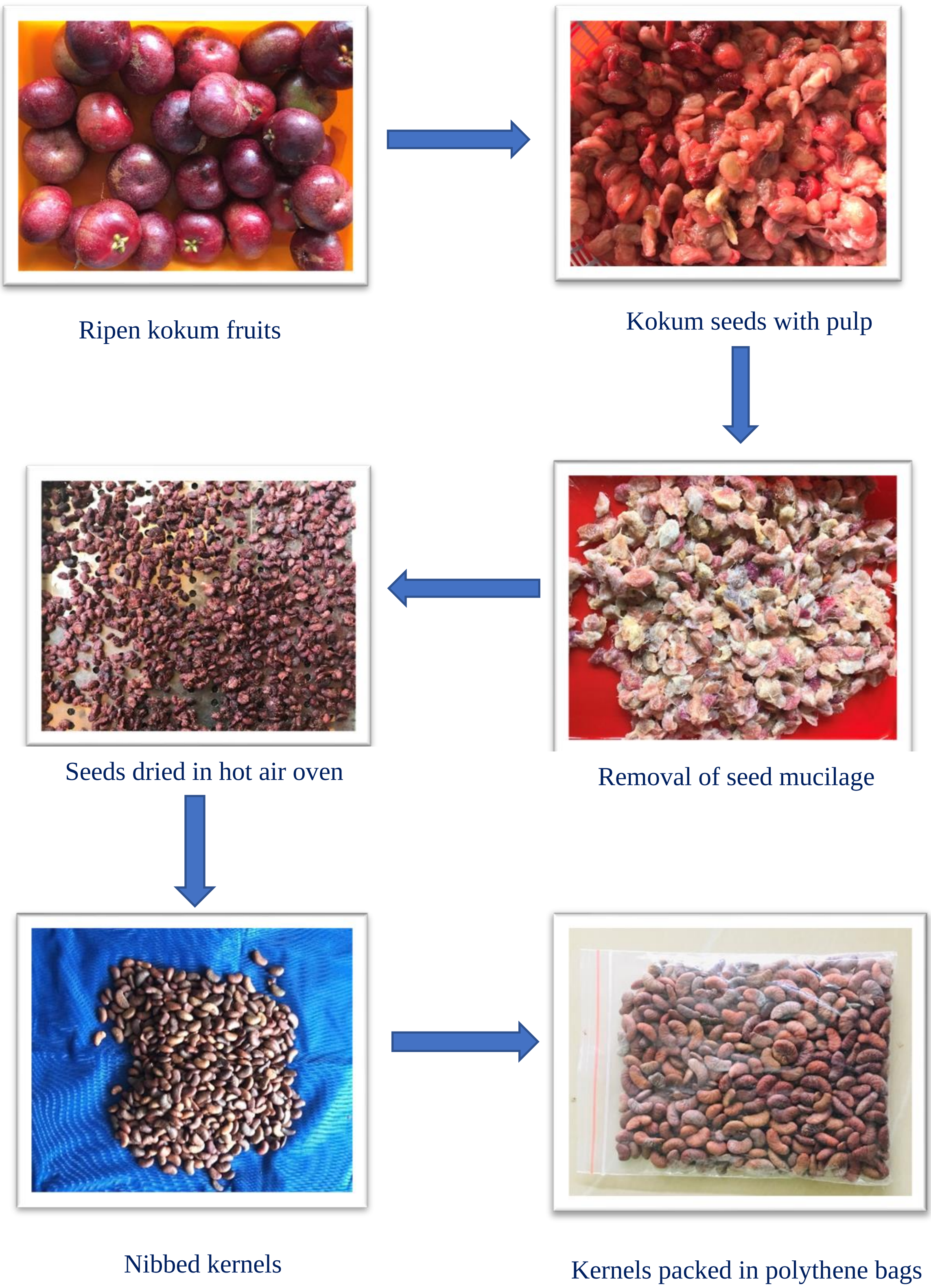


Plate 3.5. Steps followed for extraction of kokum seed kernels

alkaline copper solution was thoroughly mixed and left to stand for 10 minutes. Folin-Ciocalteau reagent of 0.5 ml was added and incubated at room temperature in the dark for 30 minutes and the generated blue colour was measured at 660 nm. A standard graph was drawn using successive dilutions of alkaline copper solution and Folin Ciocalteau reagent. The total amount of protein in the sample was determined and expressed in grams per 100 g of sample comparing the standard graph.

3.2.5. Total fat (%)

The fat content of cambodge and kokum seed was determined following the AOAC procedure (1955). Twenty grams of seed powder was placed in a thimble and clogged with cotton. The substance was extracted with petroleum ether for 3 to 4 hours without interruption by mild heating in a Soxhlet apparatus. The extraction flask was removed and allowed to cool, and the ether was separated by heating and weight was recorded. Total fat content was reported in per cent.

$$\text{Total fat (\%)} = \frac{\text{Wt. of fat (g)}}{\text{Wt. of the sample (g)}} \times 100$$

3.2.6. Total ash or Total minerals (%)

Total ash was calculated by placing approximately five grams of the sample in a crucible (which had previously been heated to around 600°C and cooled). The crucible was put on a clay pipe triangle and heated over a low flame until all of the material was thoroughly charred, followed by placing 4 to 5 hours in a muffle furnace at 600°C. After that, it was cooled and weighed consecutively twice to record similar values and the ash was virtually white or greyish-white in appearance (AOAC, 1980).

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

3.2.7. Dietary fibre (%)

The dietary fibre in the seed powder was estimated by removing the starch and protein from the alcohol-insoluble fraction. One gram of sample was weighed and placed in a clean thimble with filter paper before being extracted for 16 hours in a soxhlet apparatus using 90% ethanol as the solvent. The insoluble alcohol remnant is then dried, weighed and ground into a fine powder before being analysed for starch and protein. The quantity of total dietary fibre (TDF) in the sample was computed using the formula (Englyst and Hudson, 1987).

$$\text{TDF (\%)} = \frac{W_1 \ (W_2 + W_3)}{W} \times 100$$

W = Weight of seed powder taken (g)

W_1 = Dry weight of alcohol insoluble residue (g)

W_2= Amount of protein present in the sample (g)

W_3= Amount of starch present in the sample (g)

3.2.8. Calcium and Iron (mg per 100 g)

Calcium and iron contents were determined by ICP- optical emission spectrophotometer technique using the diacid extract generated from the sample (Perkin and Elmer, 1982). The diacid was prepared using nitric acid and perchloric acid in a 9:4 ratio. In this diacid, one gram of seed powder was digested and the extract was prepared up to 100 ml. This solution was directly measured using an atomic absorption spectrophotometer. The calcium and iron content were stated in mg per 100 g of sample.

3.2.9. Potassium and Sodium (mg per 100 g)

Potassium and sodium contents were calculated using flame photometric techniques (Jackson, 1973). One gram of seed powder was initially digested using a 9:4 mixture of nitric acid and perchloric acid in a volume of 50 ml. The sample was measured in a flame photometer with KCl and NaCl as standards for potassium and sodium, respectively. The results were represented in mg per 100 g of sample.

3.3. Estimation, physico-chemical and nutritional analysis of butter

Estimation of butter from the select accessions of cambodge and kokum was done following standard procedures. Isolated butter (bi-product) was examined for physico-chemical, nutritional and bioactive compounds using standard operating procedures.

3.3.1. Estimation of butter

The butter recovery of cambodge and kokum accessions was done using solvents in the Soxhlet apparatus. The solvents used to estimate butter were acetone, petroleum ether and petroleum benzene.

The traditional method of extraction of cambodge and kokum butter was also done through hot water skimming or a wet process. Steps followed in the estimation of butter through the traditional method is presented in Fig. 3.2.

Seeds were separated from fresh fruits and dried separately

↓

About 200 grams of kernels were separated from dried seeds by removing the seed coat

↓

Kernels were soaked in hot water (50-60 °C) to remove the impurities or tannins present on the surface

↓

Kernels were heated gently in an open pan over low flame to release of fat

↓

Roasted kernels were finely grinded into powder using mixer

↓

Two litres of water is added in an open pan and allowed it to boil for 4-5 hours.

↓

Decanting of the oily upper layer into a clean vessel and allowed the water to evaporate

↓

Butter is a type of dried fat that is kept in a dry, airtight container

Fig. 3.2. Flowchart for the preparation of butter through traditional wet process

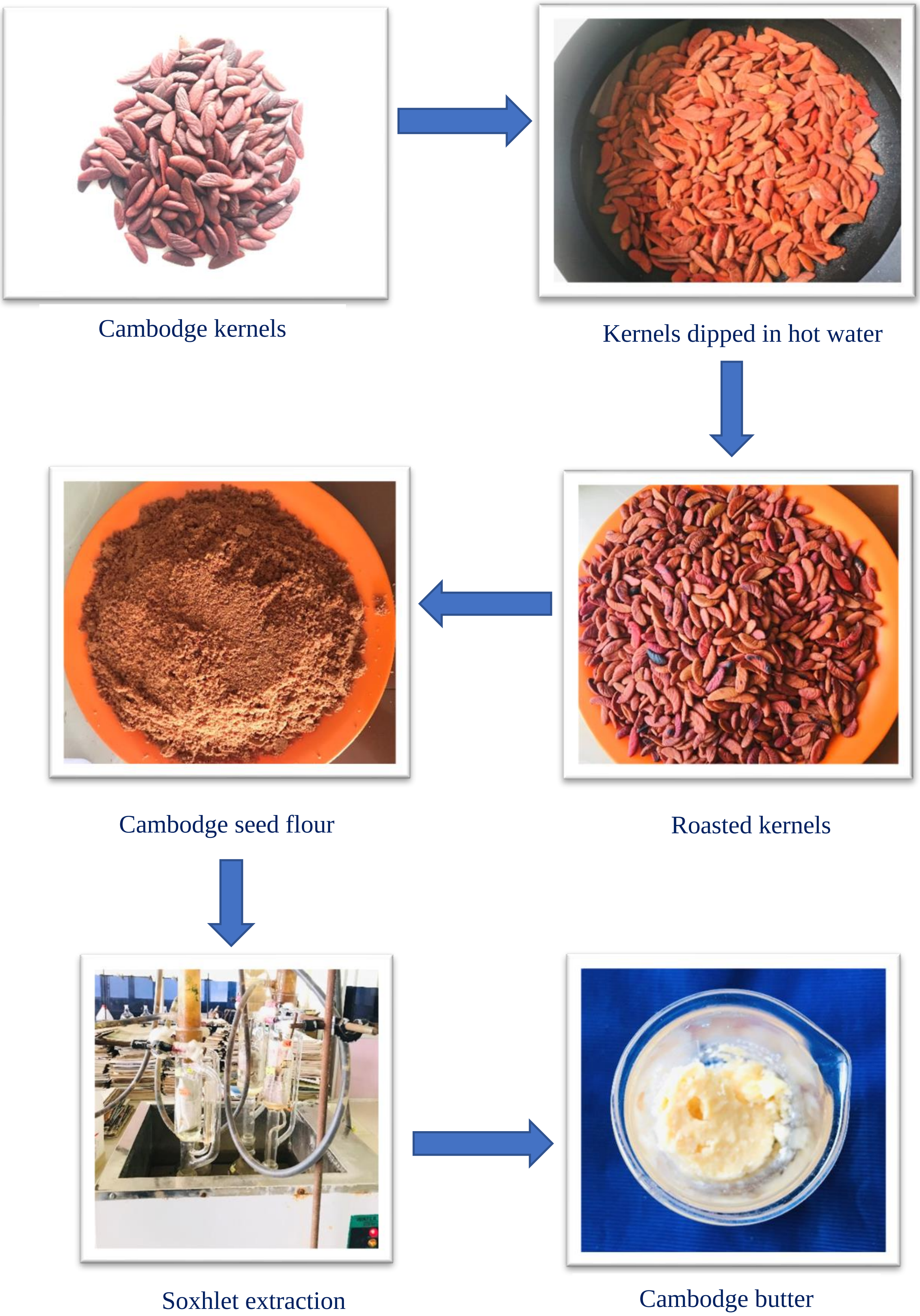


Plate 3.6. Procedure followed for extraction of butter from cambodge seed kernels

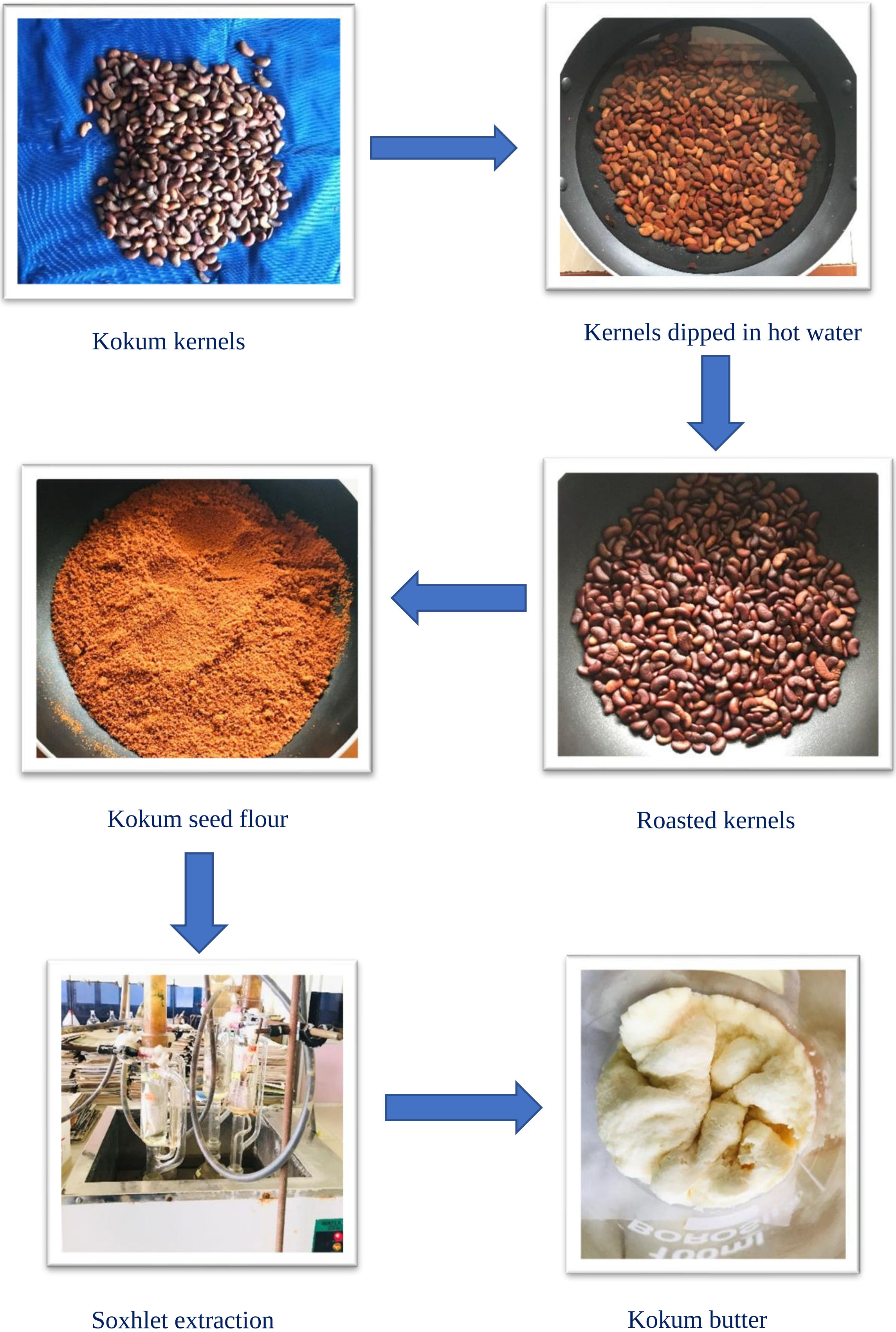


Plate 3.7. Procedure followed for extraction of butter from kokum seed kernels

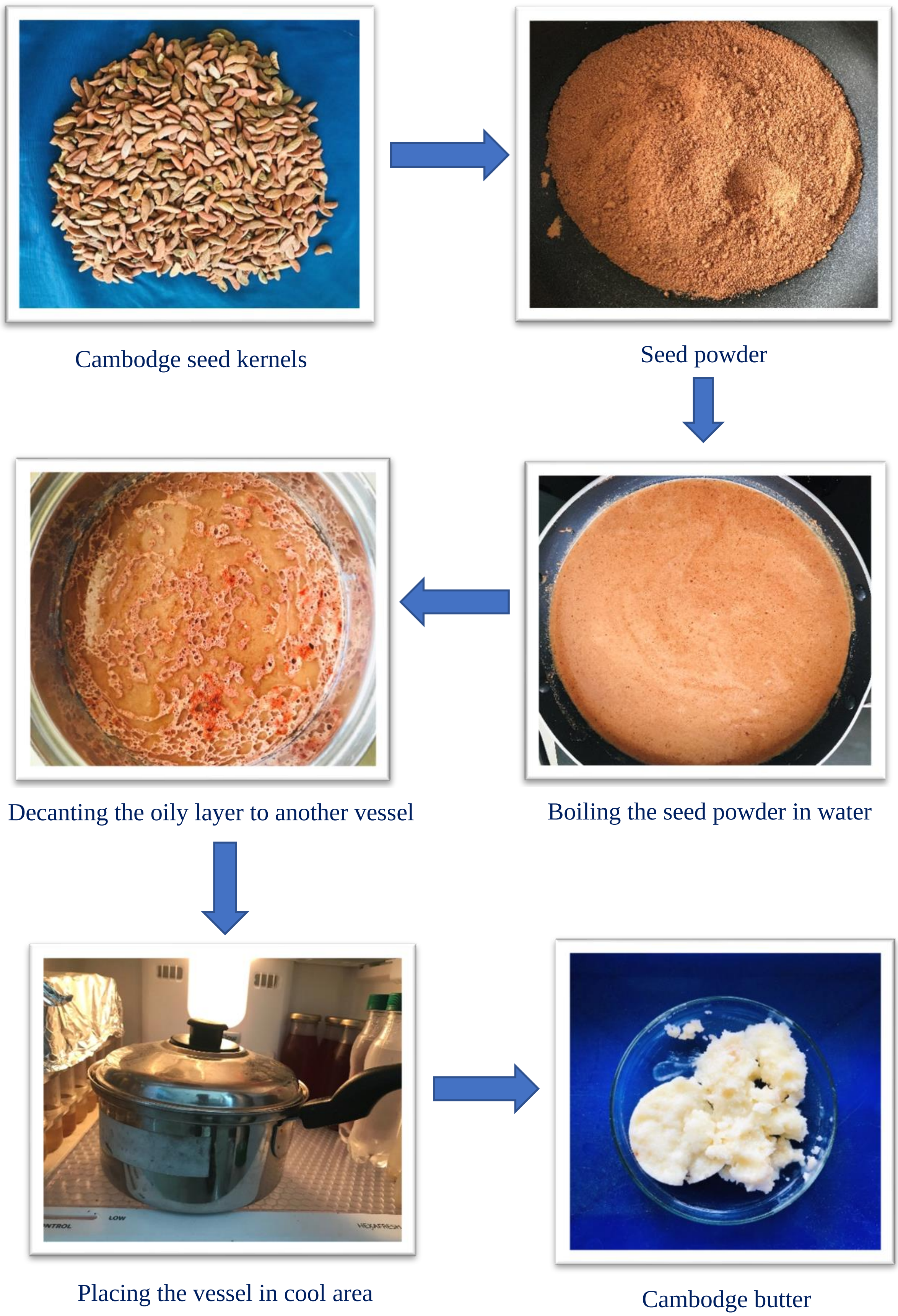


Plate 3.8. Procedure followed for traditional method of extraction of butter from cambodge seeds

Plate 3.9. Flame photometer used to measure potassium and sodium content

Plate 3.10. ICP- Optical emission spectrophotometer used to measure calcium and iron content

3.3.2. Physico-chemical analysis of seed butter

3.3.2.1. Physical state at room temperature

The physical state of cambodge and kokum butter at room temperature were determined through visual assessment.

3.3.2.2. Moisture content (%)

Moisture analyzer (Essae MX-50) was used to measure the moisture content of both cambodge and kokum butter. Five grams of each sample was placed in a moisture analyzer and the moisture content of the butter was recorded.

3.3.2.3. Oil content or Total fat (%)

Oil content of cambodge and kokum butter were determined by removing the moisture from their butter sample.

Oil content (%) = 100 – moisture content (%)

3.3.2.4. Refractive index

A conventional Abbe refractometer was used to resolve the refractive index of butter in a visible-range specimen.

3.3.2.5. Melting point (^{o}C)

It was estimated by the method given by Ranganna (1986). Both cambodge and kokum butter were heated above the melting point and a fine glass capillary tube was dipped into it to allow the sample to rise in the capillary tube. Fat was allowed to solidify for 24 hours and later, the capillary tubes were attached to the bulb of a thermometer with rubber and immersed in cold water. The temperature at which the fats started rising was noted.

3.3.2.6. Boiling point (^{o}C)

The boiling point was studied by placing 20 grams of butter in a vessel and allowed to boil on a low flame of heat. Heating was continued until the bubbles were observed. The heating was stopped when bubbles arose; the temperature was measured with the help of a thermometer and the boiling point was noticed.

3.3.2.7. Solubility

A sample of 5 grams of butter was taken separately in three test tubes. Added 5ml of water, alcohol and chloroform separately in each test tube. Shake the test tubes vigorously to check the solubility of butter in various solvents.

3.3.2.8. pH

A sample of 20 grams of butter was homogenized and pH was determined by digital pH meter.

3.3.2.9. Ash content or Total minerals (%)

Ash content of butter was determined as mentioned in the 3.2.6.

3.3.2.10. Acid value (mg KOH per g)

Five grams of butter was dissolved in 50 ml of neutral solvent, and phenolphthalein was added as an indicator and then titrated against 0.1 N potassium hydroxide. Samples were shaken until a pink colour persisted for 15 seconds. The values were expressed in mg KOH per g.

$$\text{Acid value (mg KOH per g)} = \frac{\text{Titre value X Normality of KOH}}{\text{Weight of the sample (g)}} \times 56.1$$

3.3.2.11. Saponification value (mg KOH per g)

The saponification value of cambodge and kokum seed butter was estimated using ASTM D1962 titration method (Sadasivam and Manickam, 1997). In a flask, a known amount of melted fat was mixed with 50 ml of alcoholic KOH and refluxed for 60 minutes using an air condenser. After cooling, one ml of phenolphthalein indicator was added and titrated against 0.5 N HCl until the pink colour vanished. In a similar way, blank of 50 ml of alcoholic KOH was prepared and titrated against 0.5 HCl.

$$\text{Saponification value} = \frac{(\text{Titre value of blank} - \text{titre value of sample})}{\text{Weight of the sample (g)}} \times 28.05$$

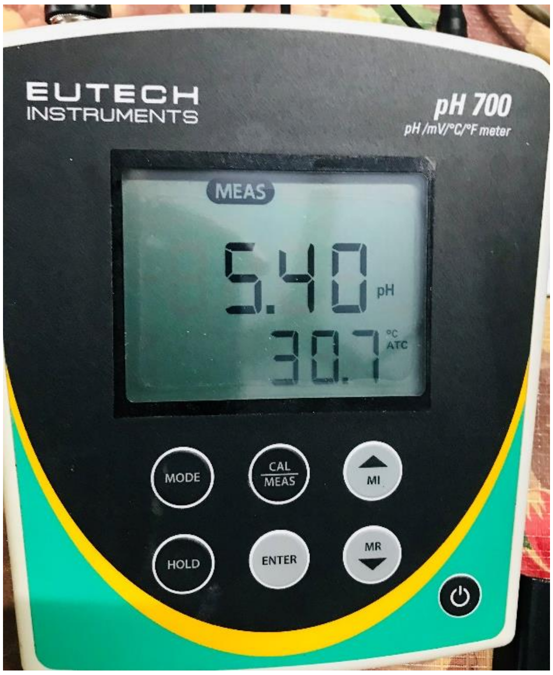


Plate 3.11. Digital pH meter used to measure the pH of *Garcinia* species butter

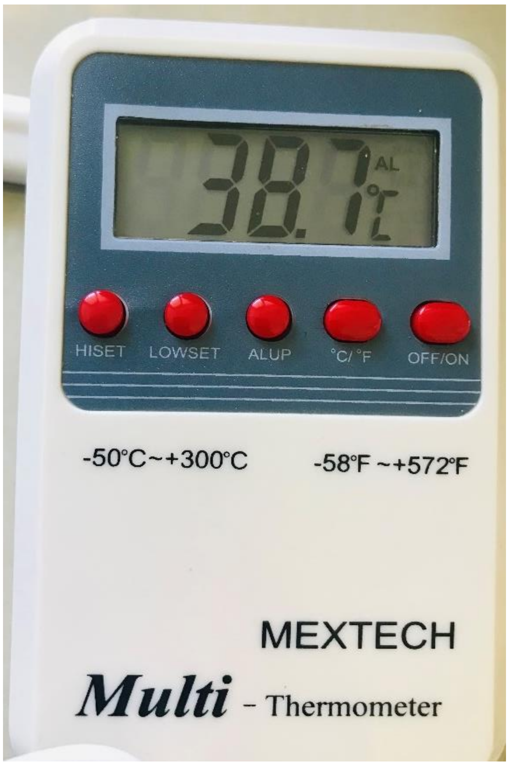


Plate 3.12. Digital thermometer used to measure boiling point (°C) of *Garcinia* species butter

3.3.2.12. Iodine value (g per 100 g)

The iodine value of cambodge and kokum butter was estimated using Wijs method (Sadasivam and Manickam, 1997). A known amount of butter was melted and combined with 10 ml CCl_4 and 25 ml Wijs solution. After 30 minutes in the dark, it was mixed with 15 ml of 10 per cent potassium iodide solution. The prepared sample was titrated with 0.1 $Na_2S_20_3$ solutions using starch as an indicator.

$$\text{Iodine value (g per 100 g)} = \frac{\text{(Blank titre} - \text{sample titre) X N of } Na_2S_20_3}{\text{Weight of sample (g)}} \text{ X } 12.66$$

3.3.2.13. Peroxide value (meq per kg)

The peroxide value of fats was calculated to determine their oxidative stability (Crowe and White, 2001). The sample (5 g) was dissolved in 30 ml of acetic acid-chloroform (3:2) solution followed by the addition of saturated potassium iodide solution (0.5ml). After a minute of thorough whirling in the dark, 30 ml of distilled water was added and the solution was titrated against 0.01 N sodium thiosulfate solution after being standardized with potassium dichromate and utilising starch as an indicator. In a similar way, blank solution was prepared and titrated against 0.01 N sodium thiosulfate.

$$\text{Peroxide value (meq per kg oil)} = \frac{(S - B) \times \text{normality of sodium thiosulfate}}{\text{Weight of the sample}} \times 1000$$

Where, S is the titration value of butter

B is the titration value of blank

3.3.2.14. Ester value (mg KOH per g)

Ester value (EV) is defined as the milligrams of KOH required to react with glycerine after saponification of one gram of lipid. It was calculated from the saponification value (SV) and acid value (AV).

$$EV = SV - AV$$

3.3.3. Nutritional analysis of butter

3.3.3.1. Calcium and Iron (mg per 100 g)

Calcium and Iron content of seed butter was estimated as mentioned in 3.2.8.

3.3.3.2. Potassium and Sodium (mg per 100 g)

Potassium and sodium content of seed butter was estimated as mentioned in 3.2.9.

3.4. GC-MS profiling of butter

Bioactive compounds are analysed through Gas chromatography techniques and the mass of the constituents was identified. The butter extracted from cambodge and kokum accessions using different solvents was used for fatty acid profiling through GC-MS technique.

3.4.1. Lipid fractionation by column chromatography

Homogenize a known weight of the sample in 10 ml of 3:1 mixture (chloroform per methanol solution). The homogenate was filtered through filter paper and the extraction was repeated three times with the same solvent. Once the extraction was completed, all of the filtrate material was collected and used a separating funnel to separate the solvent and water phases. Once separated, add 15 ml of sodium sulphate to the solvent phase. Dry the entire sample in the round bottom flask.

3.4.2. Esterification

Add one ml of BF_3 per methanol to the dry round bottom flask through a condenser with the help of a pipette and allow it to boil at 45°C - 50°C for 10 minutes. Boiling continued for about 10 minutes after adding 3 ml of heptane through the condenser. Finally, add 2 ml of heptane and set it aside to cool. Remove the conical flask from the condenser, add 2 ml of saturated NaCl solution to the conical flask, shake it well, and pour the entire contents into the test tubes after the condenser has reached room temperature. Transfer the upper heptane layer to another test tube containing sodium sulphate, then use a syringe to filter the heptane layer through nylon filter paper and inject it into the GC-MS.

3.4.3. GC-FID analysis

GC-FID analysis of fatty acid methyl esters was performed on a fused silica capillary column (VF-5 Factor Four, Lake Forest, CA, USA), 30 m × 0.25 mm id and 0.25 μm film thickness, using a Varian-3800 Gas chromatograph system equipped with a flame ionisation detector (FID). The column's temperature programme is as follows: The oven temperature is set at 100°C for 4 minutes, then increased by 3°C every minute to 220°C and held for 4 minutes. The temperature is raised to 260°C at a rate of 5°C per minute and held for 10 minutes. The temperatures of the injector and detector are kept at 250°C and 260°C, respectively. After 8 minutes, the injection is completed in split-less mode, followed by split mode (1:20).

3.4.4. Gas chromatography-mass spectrometry (GC-MS)

GC-MS analysis was carried out on a Varian-3800 gas chromatography in conjunction with a Varian 4000 GC-MS-MS ion trap mass selective detector. Fatty acids were separated on a VF-5MS fused silica capillary column (Varian, USA), (30 m 0.25 mm id with 0.25 μm film thickness) maintaing the standard temperature programme. Initially, the oven temperature was set at 100°C for 4 minutes, then increased by 3 °C per minute to 220 °C and held for 4 minutes. The temperature was further raised to 260°C at a rate of 5°C per minute and held for 10 minutes. Helium was used as the carrier gas, with a flow rate of 1ml per min, an injector temperature of 260°C, an ion source temperature of 220°C, a trap temperature of 200°C and a transfer line temperature of 260°C. EI- mode at 70 eV with full scan range, 50-450 amu were the mass detector conditions. Fatty acids were identified by comparing the relative retention durations of fatty acid methyl esters (FAME) peaks to those of reference standards (Sigma-Aldrich, USA) as well as the spectra to those accessible in the Wiley and NIST-2007 spectral libraries (Liu, 1994). The total amount of FAME was assessed as the sum of all GC-FID peak regions in the chromatogram. Individual compounds were quantified by comparing the known individual FAME obtained as standard. GC-MS results are presented in percentage.

3.5. Organoleptic evaluation of butter and value added products

Organoleptic qualities of extracted butter of both species were evaluated as well as compared with milk and cocoa butter. Milk butter was procured from the local market of Mannuthy under the brand name milky mist, whereas cocoa butter was procured from Cocoa Research Station, KAU, Vellanikkara, Thrissur. Cambodge and kokum butter were extracted traditionally through the hot water skimming method.

3.5.1. Preparation of value added products

Value added products such as burfi and cake were prepared using four types of butter. The flow chart for preparation of burfi and cake was presented in Fig. 3.3 and 3.4, respectively.

3.5.1.1. Preparation of Burfi

Ingredients: Bengal gram powder – 500 g

Jaggery – 500 g

Raisin – 25 g

Cardamom – 10 g

Almond – 50 g

Cashew – 50 g

Burfi was prepared by using following treatments.

T_1: Milk butter (250 g) + ingredients

T_2: Kokum butter (250 g) + ingredients

T_3: Cambodge butter (250 g) + ingredients

T_4: Cocoa butter (250 g) + ingredients

Prepare jaggery solution and cooked over fire until one thread consistency

↓

Add the roasted bengal gram powder flour along with the butter to a jaggery syrup and mix it well

↓

Stir well continuously until the mass separated from the sides of pan

↓

Add raisins, almond, cashew and cardamom powder at the end of cooking

↓

Switch off the flame and transfer the contents to a greased butter plate and allow to set

↓

Cut burfi into desired shapes and serve

Fig. 3.3. Flow chart for the preparation of burfi using different types of butter

3.5.1.2. Preparation of butter cake

Ingredients: All purpose flour/ Maida flour – 100 g

Baking powder – 2.5 g

Sugar – 100 g

Eggs – 2

Milk – 4 table spoons

Vanilla essence – 1 tsp

A pinch of salt

Butter cake was prepared by using following treatments.

T_1: Milk butter (125 g) + ingredients

T_2: Kokum butter (125 g) + ingredients

T_3: Cambodge butter (125 g) + ingredients

T_4: Cocoa butter (125 g) + ingredients

3.5.2. Procedure followed for organoleptic evaluation of butter and value added products

3.5.2.1. Selection of judges

An organoleptic evaluation was carried out at the laboratory level with a panel of nineteen judges between the age group of 21 to 42 years.

3.5.2.2. Preparation of score card

Score card variables of seven quality attributes *viz.* appearance, colour, flavour, texture, taste, after taste and overall acceptability was used for organoleptic evaluation of butter and their value added products. Each of the above mentioned qualities was assessed by nine point hedonic scale. Nine point hedonic scale is presented in Table 3.2.

Plate 3.13. Refractometer used to measure the refractive index of *Garcinia* species butter

Plate 3.14. Preparation of cake

Preheat oven to 180 °C and line a loaf pan with parchment paper and set aside

↓

Take maida flour, baking powder and salt in a sieve and sift it together and set aside

↓

Take butter, sugar and vanilla in a bowl and use a beater to whip it till fluffy

↓

Add eggs to the bowl and mix it well

↓

Add half of the flour and fold gently then add milk to it for proper mixing

↓

Add remaining flour and fold gently

↓

Spoon this into the pan and bake for 30 to 35 minutes (180°C)

↓

Remove it from the oven and cool it down

↓

Remove cake from the pan and allow it to cool completely

↓

Slice the cake pieces and serve

Fig. 3.4. Flow chart for the preparation of cake using different types of butter

Table 3.2. Nine point hedonic scale

Characteristics	Score card
Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

3.6. Statistical analysis

The data recorded from the study was subjected to statistical analysis. The data on biochemical, nutritional parameters of seed powder and seed butter and extraction of butter were subjected to Completely Randomized Design (CRD) analysis and T-test. CRD analysis was done to compare among the accessions of cambodge and kokum. Whereas, T-test was carried out to analyse between cambodge and kokum. The Fatty acids profiling of butter was subjected to T-test to compare between cambodge and kokum. Both CRD and T-test were done using GRAPES software. Organoleptic evaluation of butters was performed following Kendall's W test using SPSS software.

RESULTS

4.RESULTS

The present study was carried out to characterize the physico-chemical and nutritional properties of seed and seed-butter in cambodge and kokum accessions maintained at *Garcinia* block, Department of Fruit Science, College of Agriculture, Vellanikkara; Plantation and Spice farm, Department of Plantation, Spices, Medicinal and Aromatic Crops, College of Agriculture, Vellanikkara and ICAR-NBPGR, Regional Station, Thrissur. The study entitled “Physico-chemical and nutritional analysis of seed and seed-butter of *Garcinia* spp.” revealed biochemical and nutritional parameters of seed, extraction, physico-chemical and nutritional properties of butter and finally organoleptic score card for *Garcinia* butter along with milk and cocoa butter. The results obtained are presented in this chapter.

4.1. BIOCHEMICAL AND NUTRITIONAL QUALITY OF SEEDS

4.1.1. Biochemical quality of seeds

The biochemical parameters in seed powder of cambodge and kokum accessions were estimated separately. The results are presented in the Table 4.1 and 4.2.

4.1.1.1. Moisture

A significant difference was observed among the accessions of cambodge and kokum seed powder for moisture content. In cambodge, ACC.PSMA.1 (4.56 %) recorded the lowest moisture content followed by IC244101-2 (5.30 %) and ACC.FSC.C-1 (7.42 %) whereas in kokum, the lowest moisture content was in ACC.FSC.1 (10.49 %) followed by IC342302-2 (11.38 %) and IC342296-1 (12.50 %)

There was a significant difference between the cambodge and kokum seed powder for moisture content. Cambodge seed powder (5.76 %) recorded less moisture content when compared to kokum seed powder (11.45 %).

4.1.1.2. Starch

Among the accessions of cambodge, IC244101-2 (0.672 g per 100 g) recorded significantly high starch content. Both ACC.FSC.C-1 (0.448 g per 100 g) and ACC.PSMA.1 (0.450 g per 100 g) were on par with each other. Among the accessions of kokum, significantly the higher starch content was observed in IC342296-1 (0.662 g per 100 g) followed by IC342302-2 (0.560 g per 100 g) and ACC.FSC.1 (0.478 g per 100 g).

There was no significant difference observed for starch content between cambodge and kokum seeds. Starch content of cambodge seeds (0.523 g per 100 g) was comparable to kokum (0.567 g per 100 g).

4.1.1.3. Total carbohydrates

In cambodge, ACC.PSMA.1 (14.93 g per 100 g) recorded significantly highest carbohydrate content followed by IC244101-2 (14.02 g per 100 g) and ACC.FSC.C-1 (11.03 g per 100 g). Whereas in kokum, ACC.FSC.1 (5.13 g per 100 g) recorded significantly the highest carbohydrate content followed by IC342302-2 (4.52 g per 100 g) and IC342296-1 (4.03 g per 100 g).

Total carbohydrate content varied significantly in cambodge and kokum seed powder. The total carbohydrate content in seed powder of cambodge (13.33 g per 100 g) was higher than that of kokum (4.56 g per 100 g).

4.1.1.4. Protein

Among cambodge accessions, significantly high protein content was recorded in seed powder of IC244101-2 (1.39 g per 100 g) which differed substantially from ACC.FSC.C-1 (1.28 g per 100 g) and ACC.PSMA.1 (1.17 g per 100 g). Among kokum accessions, ACC.FSC.1 (0.66 g per 100 g) recorded significantly higher protein content than that of IC342302-2 (0.42 g per 100 g) and IC342296-1 (0.18 g per 100 g).

Both cambodge and kokum seed powder significantly varied for protein content. Protein content recorded in cambodge was 1.28 g per 100 g whereas, in kokum it was 0.42 g per 100 g.

4.1.1.5. Total fat

Among the cambodge accessions, significantly high total fat content was recorded in ACC.PSMA.1 (42.06 %). Both IC244101-2 (36.86 %) and ACC.FSC.C-1 (35.30 %) were on par at each other. Among the kokum accessions, significantly high total fat content was recorded in IC342296-1 (41.57 %) which was on par with IC342302-2 (39.99 %) and ACC.FSC.1 (39.27 %).

Significantly high total fat was recorded in the seed powder of kokum when compared to cambodge. Total fat of cambodge was 38.41 per cent and kokum was 40.28 per cent.

4.1.1.6. Total ash or total minerals

Among the accessions of cambodge, significant difference was observed for total ash or total mineral content. IC244101-2 (2.77 %) recorded higher total ash than that of ACC.FSC.C-1 (2.55 %) and ACC.PSMA.1 (2.26 %). Among the accessions of kokum, IC342296-1 (2.42 %) recorded higher total ash content. IC342302-2 (2.35 %) and ACC.FSC.1 (2.30 %) were on par each other.

Total ash content varied significantly between cambodge and kokum seed powder. Cambodge seed powder (2.53 %) recorded high amount of total ash or total mineral content when compared to kokum seed powder (2.36 %).

4.1.1.7. Dietary fibre

Among the accessions of cambodge, IC244101-2 (1.74 %) recorded significantly high dietary fibre followed by ACC.PSMA.1 (1.52 %) and ACC.FSC.C-1 (1.45 %). Among the accessions of kokum, ACC.FSC.1 (4.64 %) recorded significantly high dietary fibre whereas, IC342302-2 (4.52 %) and IC342296-1 (4.50 %) were on par.

The amount of dietary fibre varied significantly in seed powder of cambodge and kokum. Dietary fibre of kokum (4.55 %) was recorded higher than of cambodge (1.57 %).

Table 4.1. Biochemical parameters of seed powder among the accessions of cambodge and kokum

Crop	Accessions	Moisture (%)	Starch (g/100 g)	Total carbohydrates (g/100 g)	Protein (g/100 g)	Total fat (%)	Total ash/ Total minerals (%)	Dietary fibre (%)
Cambodge	ACC.FSC.C-1	7.42^{a}	0.448^{b}	11.03^{c}	1.28^{b}	35.30^{b}	2.55^{b}	1.45^{c}
	ACC.PSMA.1	4.56^{c}	0.450^{b}	14.93^{a}	1.17^{c}	42.06^{a}	2.26^{c}	1.52^{b}
	IC244101-2	5.30^{b}	0.672^{a}	14.02^{b}	1.39^{a}	36.86^{b}	2.77^{a}	1.74^{a}
	SE(m)	0.02	0.01	0.11	0.01	0.73	0.02	0.01
	C.D. @5%	0.07	0.02	0.35	0.03	2.26	0.05	0.03
	C.V. (%)	0.84	2.18	1.93	1.47	4.31	1.58	1.49
Kokum	ACC.FSC.1	10.49^{c}	0.478^{c}	5.13^{a}	0.66^{a}	39.27^{a}	2.30^{c}	4.64^{a}
	IC342296-1	12.50^{a}	0.662^{a}	4.03^{c}	0.18^{c}	41.57^{a}	2.42^{a}	4.50^{b}
	IC342302-2	11.38^{b}	0.560^{b}	4.52^{b}	0.42^{b}	39.99^{a}	2.35^{b}	4.52^{b}
	SE(m)	0.08	0.01	0.08	0.01	0.63	0.01	0.02
	C.D. @5%	0.25	0.02	0.23	0.03	NS	0.02	0.07
	C.V. (%)	1.60	2.86	3.73	5.56	3.50	0.54	1.17

Superscripts with same alphabets in column represents no significant difference at the 5% level.

*NS- Non significant.

Table 4.2. Variation in biochemical characters of seed powder between cambodge and kokum

Parameters	Cambodge			Kokum			T value	P value
	Mean	SE (m)	C.V. (%)	Mean	SE (m)	C.V. (%)		
Moisture content (%)	5.76[b]	0.43	0.22	11.45[a]	0.30	0.08	-14.5	0
Starch (g/100 g)	0.523[a]	0.03	0.21	0.567[a]	0.02	0.14	-1.24	0.22
Total carbohydrates (g/100 g)	13.33[a]	0.45	0.13	4.56[b]	0.13	0.11	18.70	0
Protein (g/100 g)	1.28[a]	0.02	0.07	0.42[b]	0.05	0.48	15.00	0
Total fat (%)	38.41[b]	0.87	0.09	40.28[a]	0.42	0.04	-2.29	0.03
Total ash/ Total minerals (%)	2.53[a]	0.07	0.09	2.36[b]	0.02	0.02	2.93	0.006
Dietary fibre (%)	1.57[b]	0.03	0.08	4.55[a]	0.02	0.02	-75.2	0

Superscripts with same alphabets in row represents no significant difference at the 5% level.

4.1.2. Nutritional composition of seeds

The nutritional composition of seed powder in cambodge and kokum accessions were estimated separately. The results are presented in the Table 4.3. and 4.4.

4.1.2.1. Calcium

In cambodge, IC244101-2 (176.70 mg per 100 g) recorded significantly the highest calcium content, which was followed by ACC.FSC.C-1 (165.10 mg per 100 g) and ACC.PSMA.1 (147.80 mg per 100 g). In case of kokum seed powder, significantly the highest calcium content was observed in IC342296-1 (50.50 mg per 100 g), which was on par with IC342302-2 (48.60 mg per 100 g).

Significant difference was observed among the cambodge and kokum seed powder for calcium content. Cambodge seed powder (163.20 mg per 100 g) recorded high amount of calcium than that of kokum seed powder (48.30 mg per 100 g).

4.1.2.2. Iron

Both cambodge and kokum did not vary significantly for iron content. The iron content of cambodge seed powder ranged from 15.30 (ACC.FSC.C-1) to 16.80 mg per 100 g (ACC.PSMA.1) whereas in kokum, it ranged from 13.90 (ACC.FSC.1) to 14.70 mg per 100 g (IC342296-1).

Significant difference for iron was recorded for cambodge and kokum seed powder. Iron content of cambodge (16.20 mg per 100 g) recorded higher when compared to kokum (14.30 mg per 100 g).

4.1.2.3. Potassium

Among the accessions of cambodge, considerable difference was recorded for potassium content of seed powder. IC244101-2 (514.60 mg per 100 g) recorded significantly high amount of potassium than that of ACC.FSC.C-1 (478.70 mg per 100 g) and ACC.PSMA.1 (442.00 mg per 100 g). In case of kokum accessions, IC342296-1 (652.70 mg per 100 g) was recorded significantly high amount of potassium than that of IC342302-2 (604.90 mg per 100 g) and ACC.FSC.1 (568.00 mg per 100 g).

Potassium content varied significantly in both cambodge and kokum seed powder. Potassium content was highest in kokum seed powder (608.50 mg per 100 g) when compared to cambodge seed powder (478.40 mg per 100 g).

4.1.2.4. Sodium

Among the accessions of cambodge, IC244101-2 (16.60 mg per 100 g) significantly differed for sodium content whereas, ACC.FSC.C-1 (14.40 mg per 100 g) and ACC.PSMA.1 (14.00 mg per 100 g) were on par with each other. Among kokum accessions, IC342296-1 (35.30 mg per 100 g) was significantly higher in sodium content than that of IC342302-2 (31.80 mg per 100 g) and ACC.FSC.1 (29.10 mg per 100 g).

Significant difference was observed for sodium content between cambodge and kokum. Sodium content was significantly high in kokum seed powder (32.10 mg per 100 g) when compared to cambodge seed powder (15.00 mg per 100 g).

Table 4.3. Nutritional composition of seed powder among the accessions of cambodge and kokum

Crop	Accessions	Calcium (mg/100 g)	Iron (mg/100 g)	Potassium (mg/100 g)	Sodium (mg/100 g)
Cambodge	ACC.FSC.C-1	165.10[b]	15.30[a]	478.70[b]	14.40[b]
	ACC.PSMA.1	147.80[c]	16.80[a]	442.00[c]	14.00[b]
	IC244101-2	176.70[a]	16.50[a]	514.60[a]	16.60[a]
	SE(m)	3.29	0.59	3.76	0.26
	C.D. @5%	10.15	NS	11.58	0.81
	C.V. (%)	4.51	8.172	1.76	3.93
Kokum	ACC.FSC.1	45.90[b]	13.90[a]	568.00[c]	29.1[c]
	IC342296-1	50.50[a]	14.70[a]	652.70[a]	35.3[a]
	IC342302-2	48.60[a]	14.30[a]	604.90[b]	31.8[b]
	SE(m)	0.78	0.68	6.61	0.47
	C.D. @5%	2.41	NS	20.37	1.43
	C.V. (%)	3.61	10.61	2.43	3.25

Means in the same column having the same alphabets are not significantly different at the 5% level.

*NS- Non significant

Table 4.4. Nutritional composition of seed powder in cambodge and kokum

Parameters	Cambodge			Kokum			T value	P value
	Mean	SE (m)	C.V. (%)	Mean	SE (m)	C.V. (%)		
Calcium (mg/100 g)	163.20[a]	3.63	0.09	48.30[b]	0.66	0.05	31.15	0
Iron (mg/100 g)	16.20[a]	0.36	0.09	14.30[b]	0.38	0.10	3.62	0.001
Potassium (mg/100 g)	478.40[b]	8.18	0.07	608.50[a]	9.92	0.06	-10.12	0
Sodium (mg/100 g)	15.00[b]	0.34	0.09	32.10[a]	0.73	0.09	-21.24	0

Means in the same row having the same alphabets are not significantly different at the 5% level

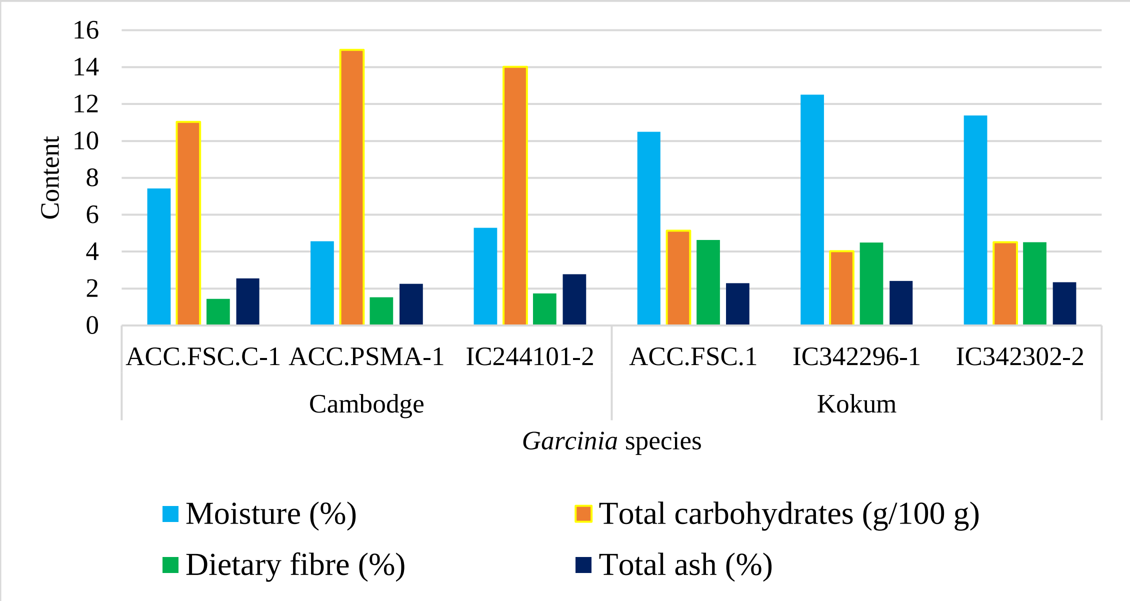


Fig. 4.1. Biochemical parameters in seed powder of cambodge and kokum accessions

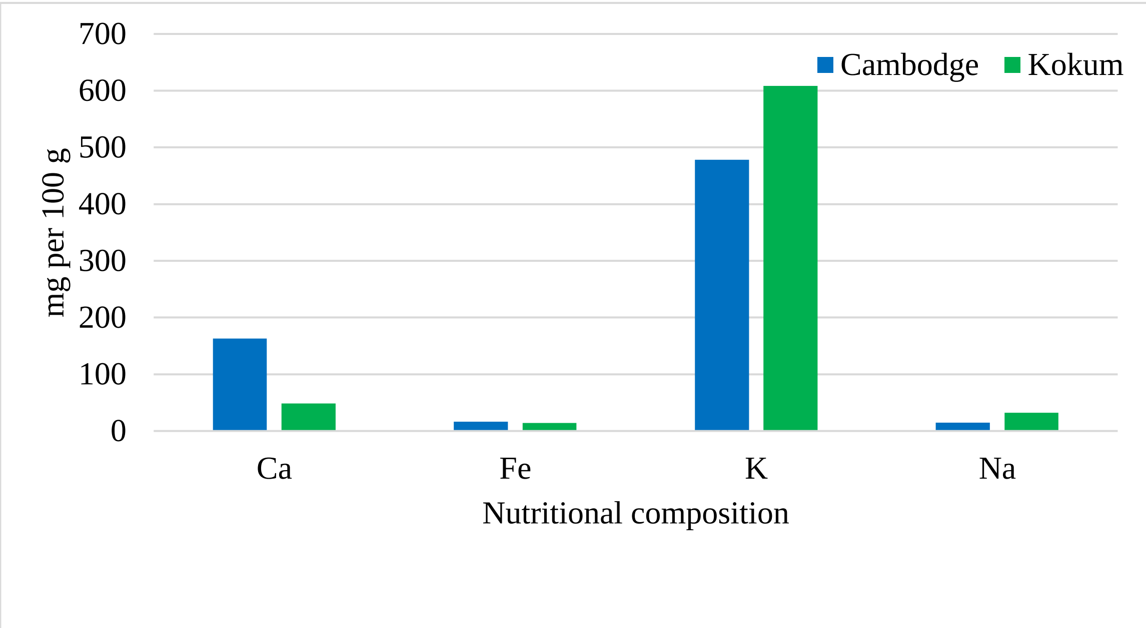


Fig. 4.2. Nutritional composition of seed powder in cambodge and kokum

4.2. EXTRACTION, PHYSICO-CHEMICAL AND NUTRITIONAL QUALITY AND PROFILING OF SEED BUTTER

4.2.1. Extraction of butter

4.2.1.1. Percentage recovery of seed butter among the accessions of cambodge and kokum

The results are presented in the Table 4.5. In cambodge accessions, significant difference was recorded in percentage of butter extracted with acetone, petroleum ether and petroleum benzene. Butter extracted with acetone recorded significantly higher in ACC.FSC.C-1 (47.19 %) followed by ACC.PSMA.1 (44.49 %) and IC244101-2 (43.26 %). In case of butter extracted with petroleum ether, significantly higher percentage recovery was obtained for ACC.PSMA.1 (42.01 %) which was followed by IC244101-2 (36.86 %) and ACC.FSC.C-1 (36.30 %). With respect to the butter extracted with petroleum benzene, ACC.PSMA.1 (41.32 %) recorded significantly higher recovery percentage than that of IC244101-2 (36.56 %) and ACC.FSC.C-1 (33.86 %).

Among the accessions of kokum, there was no significant difference in percentage recovery of butter extracted using acetone, petroleum ether and petroleum benzene. Using acetone, ACC.FSC.1 (50.67 %) was recorded higher when compared to IC342296-1 (48.88 %) and IC342302-2 (49.28 %). IC342296-1 (41.57 %) recorded high recovery of butter using petroleum ether followed by IC342302-2 (40.01 %) and ACC.FSC.1 (39.27 %). With respect to percentage recovery of butter using petroleum benzene, IC342296-1 (42.67 %) was higher than that of ACC.FSC.1 (42.11 %) and IC342302-2 (41.96 %).

4.2.1.2. Percentage of butter in cambodge and kokum using different solvents

The results for extraction of butter from cambodge and kokum using different solvents are depicted in the Table 4.6. Significant difference in the percentage recovery of seed butter was recorded in cambodge and kokum using three solvents namely acetone, petroleum ether and petroleum benzene. Using acetone, significantly higher recovery of butter was obtained in kokum (49.61 %) when compared to cambodge (44.98 %). Butter extracted using petroleum ether was observed significantly higher percentage recovery in kokum (40.29 %) when compared to cambodge (38.41 %). Significantly higher recovery

Cambodge butter

Kokum butter

Acetone

Cambodge butter

Kokum butter

Petroleum ether

Cambodge butter

Kokum butter

Petroleum benzene

Plate 4.1. Appearance of butter extracted using different solvents

of butter was recorded in kokum (42.25 %) than that of cambodge (37.25 %) seeds extracted using petroleum benzene.

The traditional extraction of butter through hot water skimming method in both cambodge and kokum seeds ranged from 22 to 25 per cent.

Table 4.5. Per cent recovery of butter using different solvents in cambodge and kokum accessions

Crop	Accessions	Acetone (%)	Petroleum ether (%)	Petroleum benzene (%)
Cambodge	ACC.FSC.C-1	47.19^{a}	36.30^{b}	33.86^{c}
	ACC.PSMA.1	44.49^{ab}	42.01^{a}	41.32^{a}
	IC244101-2	43.26^{b}	36.86^{b}	36.56^{b}
	SE(m)	0.99	0.78	0.68
	C.D. @5%	3.06	2.42	2.30
	C.V. (%)	4.95	4.57	4.08
Kokum	ACC.FSC.1	50.67^{a}	39.27^{a}	42.11^{a}
	IC342296-1	48.88^{a}	41.57^{a}	42.67^{a}
	IC342302-2	49.28^{a}	40.01^{a}	41.96^{a}
	SE(m)	0.53	0.63	0.74
	C.D. @5%	NS	NS	NS
	C.V. (%)	2.39	3.50	3.90

Superscripts with same alphabets in column represents no significant difference at the 5% level.

*NS-Non significant

Table 4.6. Per cent recovery of cambodge and kokum seed butter using different solvents

Solvents	Cambodge			Kokum			T value	P value
	Mean	SE (m)	C.V. (%)	Mean	SE (m)	C.V. (%)		
Acetone (%)	44.98^{b}	0.69	0.06	49.61^{a}	0.35	0.03	-5.99	0
Petroleum ether (%)	38.41^{b}	0.81	0.08	40.29^{a}	0.42	0.04	-2.06	0.049
Petroleum benzene (%)	37.25^{b}	0.9	0.09	42.25^{a}	0.40	0.04	-5.07	0

Superscripts with same alphabets in row represents no significant difference at the 5% level.

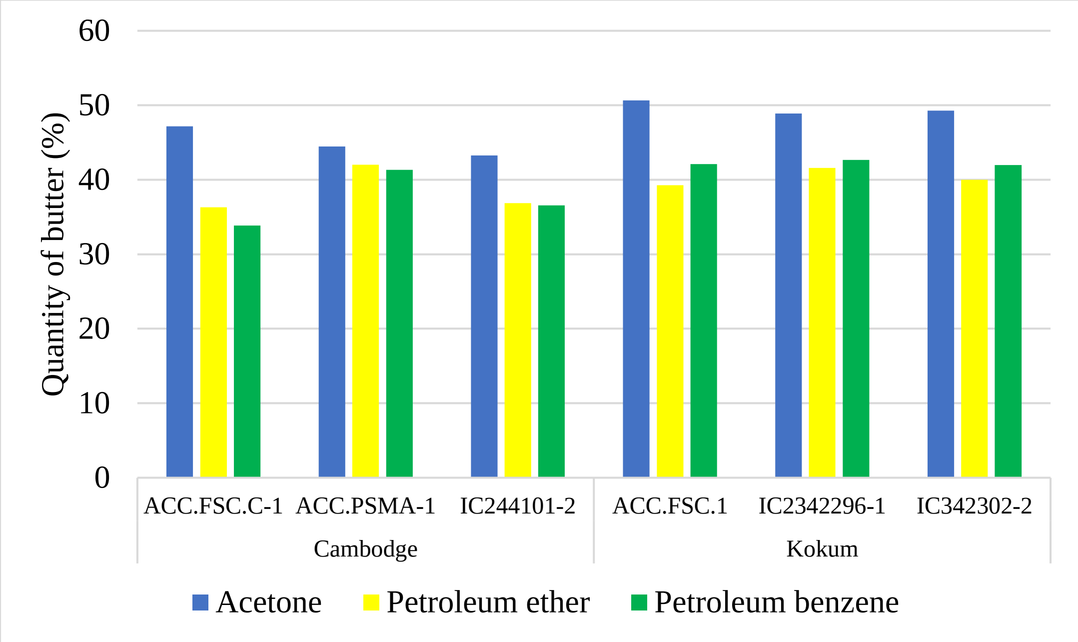


Fig. 4.3. Per cent recovery of butter using different solvents in cambodge and kokum accessions

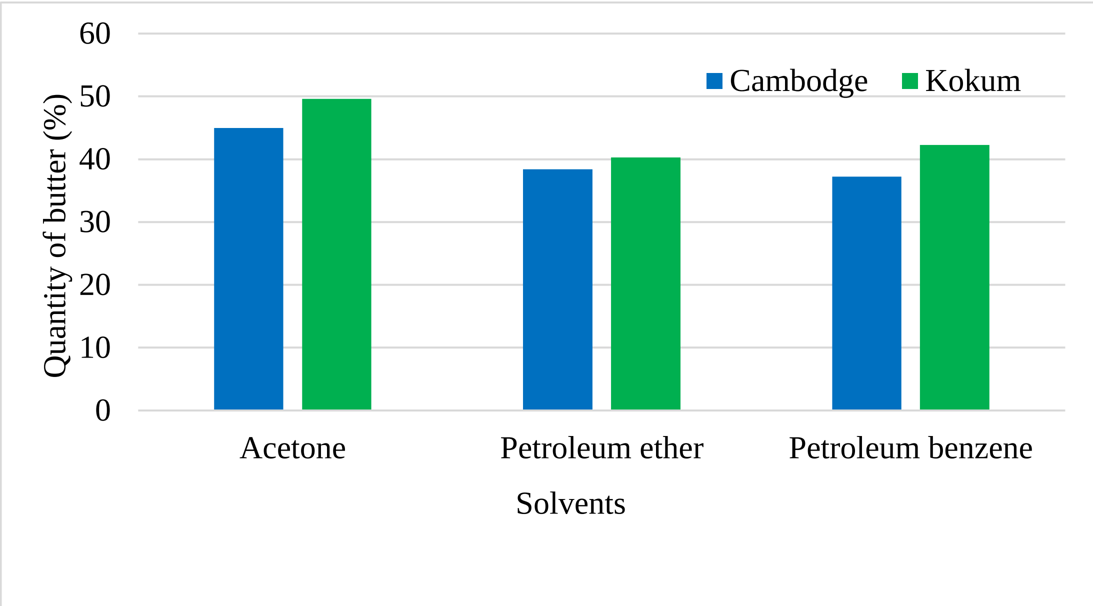


Fig. 4.4. Quantity of butter extracted using different types of solvents in cambodge and kokum

4.2.2. Physico-chemical properties of butter

The results of physico-chemical analysis of butter are depicted in Table 4.7 and 4.8.

4.2.2.1. Physical state at room temperature

The physical state of both cambodge and kokum seed butter was solid at ambient conditions.

4.2.2.2. Moisture content

In cambodge, significantly the lowest moisture content was recorded in ACC.PSMA.1 (0.19 %) when compared to ACC.FSC.C-1 (0.51 %) and IC244101-2 (0.51 %). Whereas in kokum, ACC.FSC.1 (0.11 %) was significantly lower in moisture content which was followed by IC342302-2 (0.15 %) and IC342296-1 (0.20 %).

Significant difference in moisture content of seed butter was observed in cambodge and kokum. The moisture content of kokum seed butter (0.15%) was recorded lower than that of cambodge seed butter (0.40 %).

4.2.2.3. Oil/ Total fat

In cambodge, significantly the highest oil content was recorded in ACC.PSMA.1 (99.81 %) which was followed by ACC.FSC.C-1 (99.49 %) and IC244101-2 (99.49 %). Significant difference was observed among the kokum accessions for oil content in seed butter. ACC.FSC.1 (99.89 %) recorded for higher value of oil content which was followed by IC342302-2 (99.85 %) and IC342296-1 (99.80 %).

The oil content in cambodge and kokum seed butter varied significantly. Kokum seed butter recorded highest oil content (99.85 %) than cambodge seed butter (99.60 %).

4.2.2.4. Refractive index

Among the cambodge accessions, no significant difference was observed for refractive index of seed butter. The refractive index was ranged from 1.458

(ACC.PSMA.1, IC244101-2) to 1.459 (ACC.FSC.C-1). Whereas there was significant difference among the kokum accessions for refractive index. ACC.FSC.1 (1.466) was observed significantly the highest refractive index which was followed by IC342296-1 (1.458) and IC342302-2 (1.447).

There was no significant difference for refractive index in the cambodge and kokum seed butter. The refractive index of cambodge (1.459) and kokum butter (1.457) were comparable.

4.2.2.5. Melting point

Among the accessions of cambodge, ACC.FSC.C-1 (39.62 ℃) and ACC.PSMA.1 (39.44 ℃) were recorded significantly the higher melting points followed by IC244101-2 (38.46 ℃). Among the kokum accessions, ACC.FSC.1 (39.08 ℃) recorded significantly the highest for melting point which was followed by IC342302-2 (37.83 ℃) and IC342296-1 (36.92 ℃).

The melting point of cambodge and kokum seed butter varied significantly. The melting point of cambodge seed butter (39.17 ℃) was significantly higher when compared to kokum seed butter (37.94 ℃).

4.2.2.6. Boiling point

The boiling point of butter was recorded more than 300 ℃ in all the accessions of cambodge and kokum.

4.2.2.7. Solubility

The seed butter of both cambodge and kokum were not soluble in water. Similarly, cambodge and kokum butter were also not soluble in water. Seed butter of *Garcinia* species showed a clear differentiation between water and butter as it floated on top of the water. Whereas cambodge and kokum butter showed partial soluble nature with alcohol when it is mixed vigorously. Complete solubility of kokum and cambodge butter was observed in chloroform.

4.2.2.8. pH

Among the accessions of cambodge, significant difference was observed for pH. Highest pH value was obtained in ACC.FSC.C-1 (5.69) followed by IC244101-2 (5.41) and ACC.PSMA.1 (4.97). In kokum accessions, pH value of seed butter varied significantly. ACC.FSC.1 (5.51) had the highest pH value which was followed by IC342302-2 (5.15) and IC342296-1 (4.90).

There was no significant difference observed for pH of kokum and cambodge seed butter. pH value of cambodge seed butter (5.36) was comparable to that of kokum seed butter (5.18).

4.2.2.9. Ash content or Total minerals

With respect to cambodge accessions, ACC.FSC.C-1 (0.144 %) and ACC.PSMA.1 (0.14 %) were recorded significantly the highest ash content followed by IC244101-2 (0.133 %). Kokum accessions did not vary significantly for ash content. Ash content was high in IC342296-1 (0.118 %) followed by ACC.FSC.1 (0.115 %) and IC342302-2 (0.114 %).

The ash content of cambodge and kokum seed butter varied significantly. The ash content of cambodge seed butter (0.139 %) was higher when compared to kokum seed butter (0.116 %).

4.2.2.10. Acid value

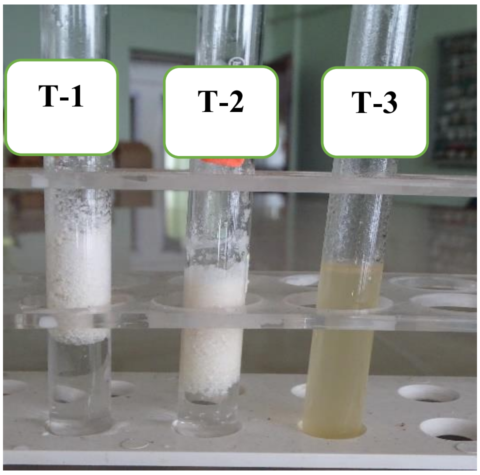
With respect to cambodge accessions, significantly the highest acid value was recorded in ACC.PSMA.1 (5.98 mg KOH per g) followed by IC244101-2 (5.80 mg KOH per g) and ACC.FSC.C-1 (5.04 mg KOH per g). In case of kokum, significantly the highest acid value was recorded in IC342296-1 (6.83 mg KOH per g) followed by IC342302-2 (5.48 mg KOH per g) and ACC.FSC.1 (4.25 mg KOH per g).

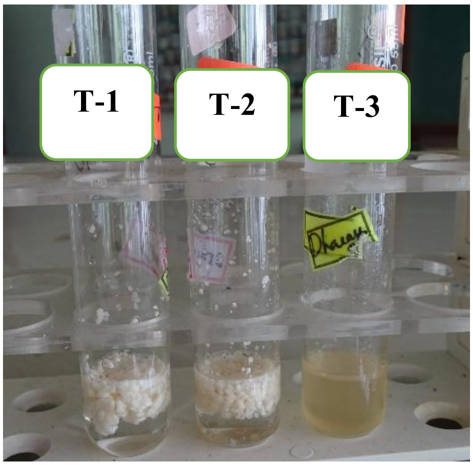
The acid value did not significantly differ between cambodge and kokum. Acid value of seed butter was recorded in cambodge (5.61 mg KOH per g) was comparable to kokum seed butter (5.52 mg KOH per g).

Kokum butter

Cambodge butter

Plate 4.2. Physical state of butter at room temperature (32 °C)





Cambodge butter

Kokum butter

T-1: Water **T-2: Alcohol** **T-3: Chloroform**

Plate 4.3. Solubility of butter in different solvents

Plate 4.4. Change in colour in titration of acid value

Plate 4.5. Change in colour in titration of saponification value

4.2.2.11. Saponification value

The saponification value of cambodge accessions differed significantly. The highest saponification value was recorded in IC244101-2 (190.60 mg KOH per g) and ACC.PSMA.1 (189.90 mg KOH per g) followed by ACC.FSC.C-1 (185.50 mg KOH per g). Whereas kokum accessions, saponification value of seed butter was significantly high in IC342296-1 (186.10 mg KOH per g) followed by IC342302-2 (179.10 mg KOH per g) and ACC.FSC.1 (174.80 mg KOH per g).

With respect to saponification value of seed butter, significant variation was observed between cambodge and kokum. Cambodge seed butter (188.70 mg KOH per g) was recorded the highest saponification value than that of kokum seed butter (180.00 mg KOH per g).

4.2.2.12. Iodine value

Cambodge accessions differed significantly for iodine value in seed butter. IC244101-2 (62.44 g per 100 g) was recorded maximum iodine value followed by ACC.PSMA.1 (55.08 g per 100 g) and ACC.FSC.C-1 (53.63 g per 100 g). Kokum accessions did not vary significantly in iodine value. Maximum iodine value was in ACC.FSC.1 (38.62 g per 100 g).

The iodine value of seed butter significantly varied between cambodge and kokum. Cambodge seed butter (57.05 g per 100 g) recorded maximum iodine value when compared to kokum seed butter (37.95 g per 100 g).

4.2.2.13. Peroxide value

The peroxide value of cambodge and kokum seed butter did not differ significantly among the accessions. Peroxide value in cambodge accessions ranged from 4.34 meq per kg (ACC.PSMA.1) to 4.44 meq per kg (ACC.FSC.C-1). Whereas peroxide value of kokum seed butter ranged from 5.77 meq per kg (IC342302-2) to 5.84 meq per kg (ACC.FSC.1).

With respect to peroxide value, cambodge and kokum exhibited significant difference. Peroxide value of cambodge seed butter (4.39 meq per kg) was lower when compared to kokum seed butter (5.81 meq per kg).

4.2.2.14. Ester value

There was no significant difference in ester value of seed butter among the accessions of cambodge. Whereas in kokum accessions, significant difference was observed. In case of kokum accessions, significantly the highest ester value was recorded in IC342296-1 (178.99 mg KOH per g) which was followed by IC342302-2 (173.61 mg KOH per g) and ACC.FSC.1 (170.76 mg KOH per g).

Ester value in cambodge and kokum seed butter varied significantly. Maximum ester value was recorded in cambodge (183.20 mg KOH per g) than that of kokum (174.40 mg KOH per g).

Table 4.7. Variation in physico-chemical parameters of seed butter of cambodge and kokum accessions

Crop	Accessions	Moisture content (%)	Oil content/ Total fat (%)	Refractive index	Melting point (℃)	Boiling point (℃)	pH
Cambodge	ACC.FSC.C-1	0.51[a]	99.49[b]	1.459[a]	39.62[a]	>300 ℃	5.69[a]
	ACC.PSMA.1	0.19[b]	99.81[a]	1.458[a]	39.44[a]	>300 ℃	4.97[c]
	IC244101-2	0.51[a]	99.49[b]	1.458[a]	38.46[b]	>300 ℃	5.41[b]
	SE(m)	0.01	0.01	0.00	0.15	-	0.02
	C.D. @5%	0.02	0.02	NS	0.46	-	0.06
	C.V. (%)	2.94	0.01	0.065	0.85	-	0.81
Kokum	ACC.FSC.1	0.11[c]	99.89[a]	1.466[a]	39.08[a]	>300 ℃	5.51[a]
	IC342296-1	0.20[a]	99.80[c]	1.450[b]	36.92[c]	>300 ℃	4.90[c]
	IC342302-2	0.15[b]	99.85[b]	1.447[c]	37.83[b]	>300 ℃	5.15[b]
	SE(m)	0.01	0.01	0.00	0.17	-	0.02
	C.D. @5%	0.01	0.01	0.01	0.53	-	0.05
	C.V. (%)	6.24	0.01	0.06	1.02	-	0.61

Superscripts with same alphabets in column represents no significant difference at the 5% level.

*NS-Non significant

Contd…

Table 4.7. Variation in physico-chemical parameters of seed butter of cambodge and kokum accessions

Crop	Accessions	Ash content/ Total minerals (%)	Acid value (mg KOH/g)	Saponification value (mg KOH/g)	Iodine value (g/100 g)	Peroxide value (meq/kg)	Ester value (mg KOH/g)
Cambodge	ACC.FSC.C-1	0.144^{a}	5.04^{b}	185.50^{b}	53.63^{b}	4.44^{a}	180.49^{a}
	ACC.PSMA.1	0.140^{a}	5.98^{a}	189.90^{a}	55.08^{b}	4.34^{a}	184.32^{a}
	IC244101-2	0.133^{b}	5.80^{a}	190.60^{a}	62.44^{a}	4.41^{a}	184.81^{a}
	SE(m)	0.002	0.24	1.07	1.14	0.07	1.26
	C.D. @5%	0.006	0.75	3.29	3.51	NS	NS
	C.V. (%)	3.014	9.75	1.26	4.47	3.55	1.54
Kokum	ACC.FSC.1	0.115^{a}	4.25^{c}	174.80^{c}	38.62^{a}	5.84^{a}	170.76^{b}
	IC342296-1	0.118^{a}	6.83^{a}	186.10^{a}	37.54^{a}	5.82^{a}	178.99^{a}
	IC342302-2	0.114^{a}	5.48^{b}	179.10^{b}	37.69^{a}	5.77^{a}	173.61^{b}
	SE(m)	0.002	0.14	1.03	0.38	0.06	1.04
	C.D. @5%	NS	0.43	3.18	NS	NS	3.21
	C.V. (%)	3.96	5.56	1.28	2.24	2.21	1.34

Superscripts with same alphabets in column represents no significant difference at the 5% level.

*NS-Non significant

Table 4.8. Variation in physico-chemical parameters between cambodge and kokum seed butter

Parameters	Cambodge			Kokum			T Value	P value
	Mean	SE (m)	C.V. (%)	Mean	SE (m)	C.V. (%)		
Moisture content (%)	0.40[a]	0.04	0.39	0.15[b]	0.009	0.24	6.00	0
Oil content/ total fat (%)	99.60[b]	0.04	0.01	99.85[a]	0.009	0.00	-6.00	0
Refractive index	1.459[a]	0.00	0.00	1.457[a]	0.002	0.00	0.80	0.43
Melting point (°C)	39.17[a]	0.16	0.02	37.94[b]	0.25	0.03	4.11	0.0003
Boiling point (°C)	>300	-	-	>300	-	-	-	-
pH	5.36[a]	0.08	0.06	5.18[a]	0.07	0.05	1.64	0.111
Ash content/ Total minerals (%)	0.139[a]	0.01	0.04	0.116[b]	0.01	0.04	11.59	0
Acid value (mg KOH/g)	5.61[a]	0.17	0.12	5.52[a]	0.29	0.20	0.25	0.801
Saponification value (mg KOH/g)	188.70[a]	0.83	0.02	180.00[b]	1.36	0.03	5.45	0
Iodine value (g/100 g)	57.05[a]	1.20	0.08	37.95[b]	0.24	0.02	15.64	0
Peroxide value (meq/kg)	4.39[b]	0.04	0.03	5.81[a]	0.03	0.02	-28.28	0
Ester value (mg KOH/g)	183.20[a]	0.85	0.02	174.40[b]	1.07	0.02	6.41	0

Superscripts with same alphabets in row represents no significant difference at the 5% level.

4.2.3. Nutritional composition of butter

The results for nutritional composition of seed butter in *Garcinia* species are presented in Tables 4.9 and 4.10.

4.2.3.1. Calcium

Significant difference was observed in cambodge accessions with respect to calcium content. Maximum calcium content was recorded in ACC.FSC.C-1 (25.20 mg per 100 g) followed by ACC.PSMA.1 (20.70 mg per 100 g) and IC244101-2 (20.40 mg per 100 g). Whereas in kokum accessions, ACC.FSC.1 (23.90 mg per 100 g) recorded significantly higher calcium content, which was followed by IC342302-2 (19.70 mg per 100 g) and IC342296-1 (16.00 mg per 100 g).

There was no significant difference observed for calcium content between cambodge and kokum seed butter. The calcium content was maximum in cambodge seed butter (22.10 mg per 100 g) when compared to kokum seed butter (19.90 mg per 100 g).

4.2.3.2. Iron

Significant difference was recorded for iron content in cambodge and kokum accessions. In cambodge accessions, maximum iron content was recorded in ACC.FSC.C-1 (15.10 mg per 100 g) which was followed by ACC.PSMA.1 (11.50 mg per 100 g) and IC244101-2 (11.40 mg per 100 g). Whereas in kokum accessions, IC342296-1 (15.60 mg per 100 g) recorded the highest value for iron content followed by IC342302-2 (12.70 mg per 100 g) and ACC.FSC.1 (10.10 mg per 100 g).

There was no significant difference for iron content in cambodge and kokum seed butter. The iron content of cambodge seed butter (12.70 mg per 100 g) was comparable to kokum seed butter (12.80 mg per 100 g).

4.2.3.3. Potassium

In cambodge, significantly the higher potassium content was recorded in ACC.PSMA.1 (35.50 mg per 100 g) which was followed by ACC.FSC.C-1 (31.30 mg per 100 g) and IC244101-2 (31.60 mg per 100 g). Whereas in kokum, ACC.FSC.1 (18.70 mg per 100 g) had significantly higher potassium content which was followed by IC342296-1 (18.20 mg per 100 g) and IC342302-2 (17.90 mg per 100 g).

Significant difference in potassium content of seed butter was observed in cambodge and kokum. The potassium content of cambodge seed butter (32.80 mg per 100 g) was recorded higher than that of kokum seed butter (18.30 mg per 100 g).

4.2.3.4. Sodium

Among the accessions of cambodge, significant difference was observed for sodium content. Significantly the highest sodium content was recorded in ACC.PSMA.1 (15.60 mg per 100 g) which was followed by ACC.FSC.C-1 (15.00 mg per 100 g) and IC244101-2 (14.30 mg per 100 g). In kokum accessions, sodium content of seed butter did not vary significantly. IC342296-1 (18.10 mg per 100 g) was observed higher value for sodium content which was followed by IC342302-2 (17.50 mg per 100 g) and ACC.FSC.1 (17.30 mg per 100 g).

There was significant difference in sodium content of cambodge and kokum seed butter. The sodium content of kokum seed butter (17.60 mg per 100 g) was significantly higher when compared to cambodge seed butter (14.90 mg per 100 g).

Table 4.9. Nutritional composition of seed butter among the cambodge and kokum accessions

Crop	Accessions	Calcium (mg/100 g)	Iron (mg/100 g)	Potassium (mg/100 g)	Sodium (mg/100 g)
Cambodge	ACC.FSC.C-1	25.20[a]	15.10[a]	31.30[b]	15.00[b]
	ACC.PSMA.1	20.70[b]	11.50[b]	35.50[a]	15.60[a]
	IC244101-2	20.40[b]	11.40[b]	31.60[b]	14.30[c]
	SE(m)	0.62	0.75	0.23	0.17
	C.D. @5%	1.91	2.32	0.72	0.52
	C.V. (%)	6.26	13.28	1.59	2.54
Kokum	ACC.FSC.1	23.90[a]	10.10[b]	18.70[a]	17.30[a]
	IC342296-1	16.00[c]	15.60[a]	18.20[b]	18.10[a]
	IC342302-2	19.70[b]	12.70[b]	17.90[b]	17.50[a]
	SE(m)	0.57	0.87	0.14	0.39
	C.D. @5%	1.77	2.68	0.42	NS
	C.V. (%)	6.47	15.23	1.66	4.90

Superscripts with same alphabets in column represents no significant difference at the 5% level.

*NS- Non significant

Table 4.10. Variation in nutritional composition between cambodge and kokum seed butter

Parameters	Cambodge			Kokum			T value	P value
	Mean	SE (m)	C.V. (%)	Mean	SE (m)	C.V. (%)		
Calcium (mg/100 g)	22.10[a]	0.66	0.12	19.90[a]	0.92	0.18	1.98	0.05
Iron (mg/100 g)	12.70[a]	0.61	0.18	12.80[a]	0.76	0.23	-0.14	0.88
Potassium (mg/100 g)	32.80[a]	0.52	0.06	18.30[b]	0.12	0.03	27.16	0
Sodium (mg/100 g)	14.90[b]	0.17	0.04	17.60[a]	0.23	0.05	-9.35	0

Superscripts with same alphabets in row represents no significant difference at the 5% level.

4.3. Fatty acid profiling of seed butter of cambodge and kokum

The fatty acids profiling of cambodge and kokum seed butter was done through Gas Chromatography Mass Spectrometry (GCMS). The chemical profiling of seed butter exhibited six prime compounds with difference in peak observed for different accessions as well as different solvents. The major fatty acids detected were stearic acid, oleic acid, palmitic acid, myristic acid, lauric acid and capric acid. The concentration of each fatty acid present in cambodge and kokum butter is depicted in the Table 4.11.

In cambodge, stearic acid (50.761 %) was recorded highest in seed butter extracted using petroleum ether for ACC.FSC.C-1 whereas, oleic acid (61.372 %) was recorded higher in butter extracted using acetone from ACC.PSMA.1. With respect to acetone, IC244101-2 was found highest in palmitic acid (3.056 %), lauric acid (0.141 %) and capric acid (0.115 %). Myristic acid (0.398 %) was highest in butter extracted from ACC.FSC.C-1 using acetone.

With respect to kokum, stearic acid (62.524 %) was highest in butter extracted using petroleum benzene from ACC.FSC.1 whereas, oleic acid (65.427 %) was found highest in butter extracted with acetone of ACC.FSC.1. Butter extracted from IC342296-1 using petroleum ether had high concentration of palmitic acid (4.544 %), myristic acid (0.893 %), lauric acid (0.554 %) and capric acid (0.662 %).

4.3.1. Comparision of fatty acids in cambodge and kokum using different solvents

The variation in fatty acid content between cambodge and kokum butter using different solvents is depicted in the Table 4.12. Significant variation was observed in stearic acid content of butter extracted with petroleum benzene. Kokum butter (58.08 %) was significantly higher in stearic acid when compared to cambodge butter (45.10 %) extracted using petroleum benzene. Butter extracted using acetone and petroleum ether also recorded higher stearic acid content in kokum butter (42.56 %, 55.74 %, respectively) when compared to cambodge butter (37.34 %, 49.70 %, respectively).

In case of oleic acid content, significant difference was observed in butter extracted using petroleum ether and petroleum benzene. In case of butter extracted with petroleum ether, significantly higher content of oleic acid was recorded in cambodge butter (48.12 %) than that of kokum butter (39.93 %). With respect to butter extracted using petroleum benzene, significantly higher oleic acid content was observed in cambodge butter (52.65 %) than that of kokum butter (39.50 %). There was no significant difference observed in oleic acid content of butter extracted with acetone. Cambodge butter (59.45 %) was recorded higher value than kokum butter (54.42 %).

There was no significant difference observed for palmitic acid, myristic acid, lauric acid and capric acid concentration in butter extracted using different solvents. With respect to petroleum ether and petroleum benzene, palmitic acid was recorded higher in kokum butter (3.212 & 2.258, respectively) when compared cambodge butter (1.981 & 1.682, respectively).

4.4. ORGANOLEPTIC EVALUATION

The organoleptic evaluation of various sources of butter including milk, kokum, cambodge and cocoa butter was done. The products such as burfi and cake were prepared using these butters were assessed organoleptically using nine point hedonic scale.

4.4.1. Organoleptic qualities of different butters

The mean rank score and mean score computed for quality parameters for different types of butter is presented in Table 4.13.

For the appearance of different butters, the mean score and mean rank score varied from 6.82 (cocoa butter) to 8.64 (milk butter) and 1.40 (cocoa butter) to 3.90 (milk butter), respectively. Next to milk butter, the highest mean score and mean rank score was recorded in kokum butter (7.69 & 2.43, respectively) followed by cambodge butter (7.53 & 2.27, respectively).

The mean score for colour of different butters ranged from 6.64 (cocoa butter) to 8.49 (milk butter) whereas mean rank score ranged from 1.27 (cocoa butter) to 3.63 (milk butter). The highest mean rank score was obtained in milk butter (3.63) followed by cambodge butter (2.60), kokum butter (2.50) and cocoa butter (1.27).

For flavour of different butters, the mean rank score ranged from 1.44 to 3.72. Mean rank score was higher in milk butter (3.72) followed by cocoa butter (3.03), kokum butter (1.81) and cambodge butter (1.44). Milk butter (8.73) had the highest mean score followed by cocoa butter (8.10), kokum butter (7.16) and cambodge butter (6.89).

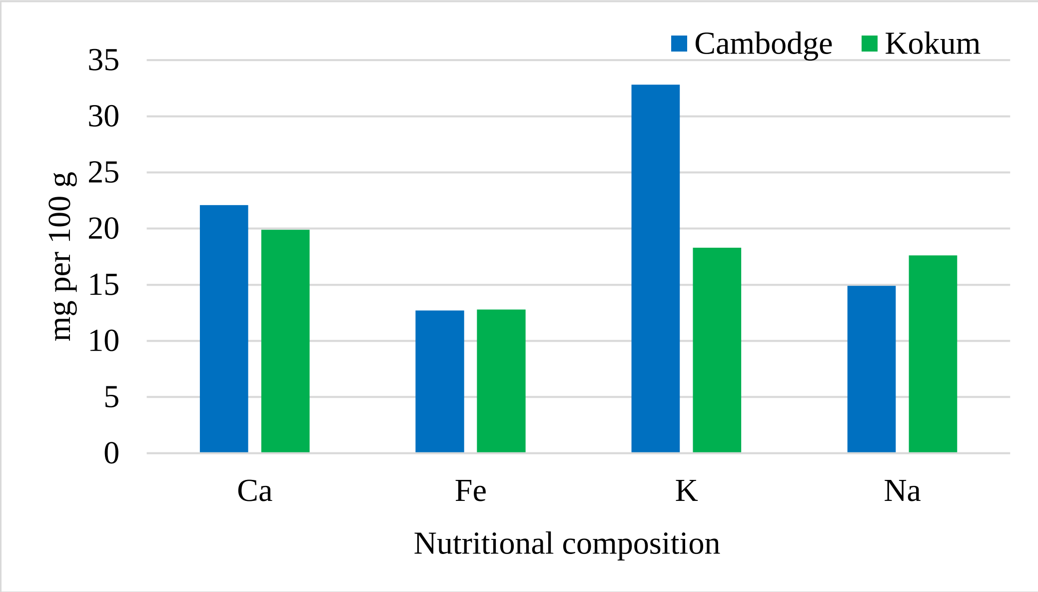


Fig .4.5. Nutritional composition of seed butter in cambodge and kokum

Table 4.11. Fatty acid composition (%) of cambodge and kokum butter

Crop	Accessions	Solvents	Capric acid/ decanoic acid	Lauric acid/ dodecanoic acid	Myristic acid/ Tetradecano ate	Palmitic acid/ hexadecanoic acid	Oleic acid	Stearic acid
Cambodge	ACC.FSC.C-1	Acetone	0.065	0.032	0.398	2.884	57.503	39.118
		Petroleum ether	0.022	0.012	0.221	2.708	46.276	50.761
		Petroleum benzene	0.003	0.003	0.011	0.760	53.198	44.726
	ACC.PSMA.1	Acetone	0.044	0.055	0.091	2.380	61.372	36.057
		Petroleum ether	0.012	0.011	0.034	1.565	48.350	50.028
		Petroleum benzene	0.025	0.037	0.077	1.781	52.148	45.932
	IC244101-2	Acetone	0.115	0.141	0.357	3.056	59.477	36.853
		Petroleum ether	0.055	0.047	0.176	1.671	49.740	48.311
		Petroleum benzene	0.074	0.057	0.097	2.504	52.617	44.651
Kokum	ACC.FSC.1	Acetone	0.049	0.047	0.080	2.341	65.427	32.056
		Petroleum ether	0.030	0.044	0.052	1.901	38.591	59.382
		Petroleum benzene	0.018	0.022	0.030	1.814	35.593	62.524
	IC342296-1	Acetone	0.177	0.094	0.251	3.014	43.784	52.679
		Petroleum ether	0.662	0.554	0.893	4.544	41.540	51.808
		Petroleum benzene	0.040	0.053	0.080	2.717	43.347	53.764
	IC342302-2	Acetone	0.112	0.070	0.164	2.651	54.059	42.944
		Petroleum ether	0.342	0.296	0.467	3.190	39.664	56.039
		Petroleum benzene	0.029	0.037	0.054	2.243	39.575	57.962

Table 4.12. Variation in fatty acid content of seed butter of cambodge and kokum extracted using different solvents

Fatty acid composition (%)	Acetone		T value	P value	Petroleum ether		T value	P value	Petroleum benzene		T value	P value
	Cambodge	Kokum			Cambodge	Kokum			Cambodge	Kokum		
Stearic acid	37.34[a]	42.56[a]	-0.86	0.43	49.70[a]	55.74[a]	-2.61	0.06	45.10[b]	58.08[a]	-5.06	0.01
Oleic acid	59.45[a]	54.42[a]	0.79	0.44	48.12[a]	39.93[b]	6.18	0.01	52.65[a]	39.50[b]	5.82	0.01
Palmitic acid/ hexadecanoic acid	2.77[a]	2.67[a]	0.37	0.72	1.981[a]	3.212[a]	-1.45	0.22	1.682[a]	2.258[a]	-1.01	0.37
Myristic acid/ Tetradecanoate	0.282[a]	0.165[a]	1.08	0.34	0.144[a]	0.471[a]	-1.31	0.26	0.062[a]	0.055[a]	0.23	0.82
Lauric acid/ dodecanoic acid	0.076[a]	0.070[a]	0.15	0.88	0.023[a]	0.298[a]	-1.86	0.14	0.032[a]	0.037[a]	-0.27	0.79
Capric acid/ decanoic acid	0.075[a]	0.113[a]	-0.89	0.42	0.030[a]	0.345[a]	-1.72	0.16	0.034[a]	0.029[a]	0.22	0.83

Means in the same row having the same letters are not significantly different at the 5% level.

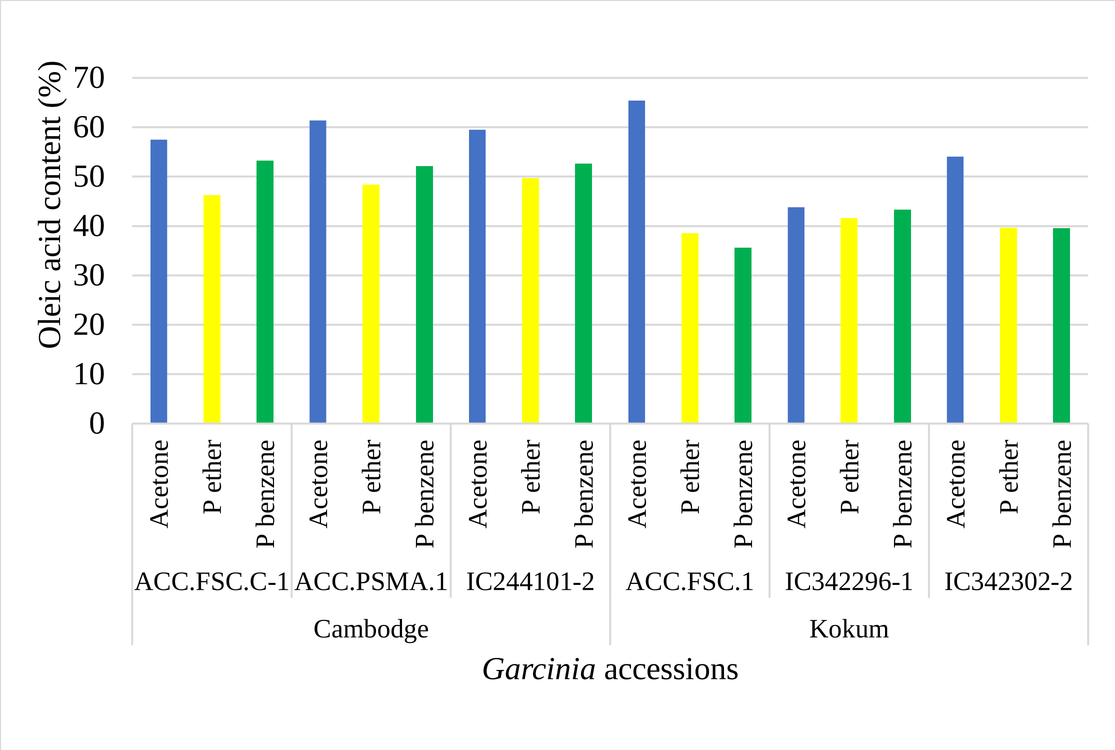


Fig. 4.6. Variation of oleic acid content in *Garcinia* species butter

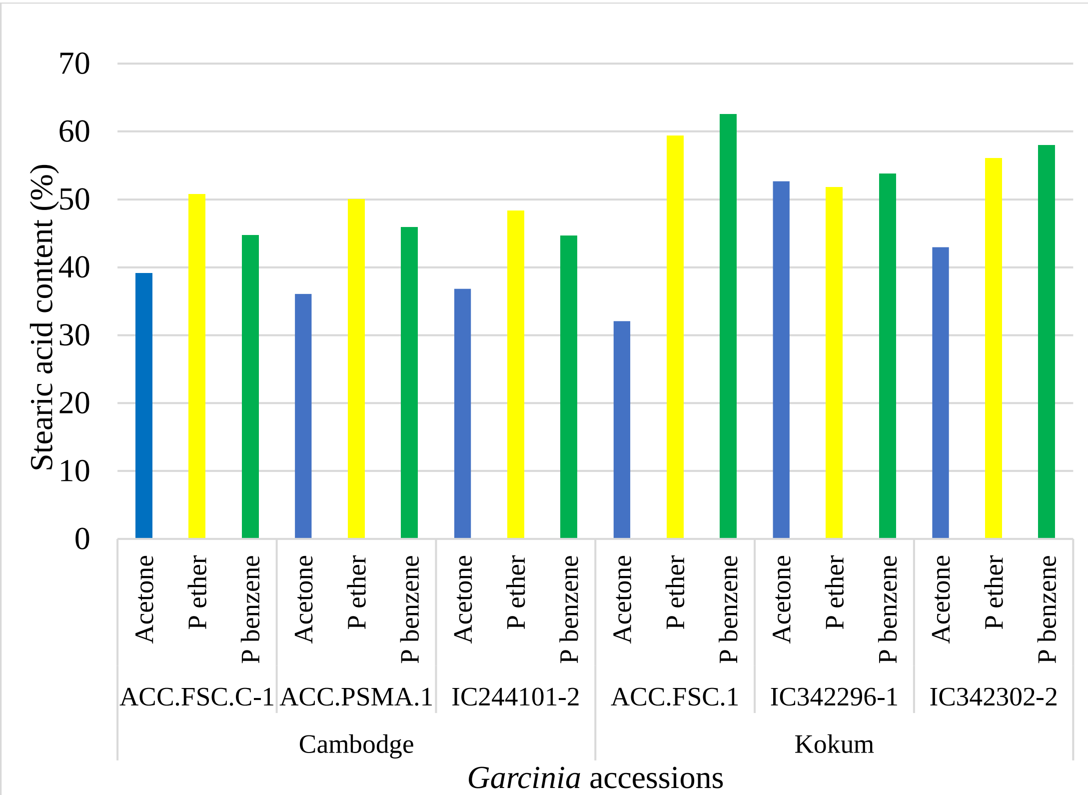


Fig. 4.7. Variation of stearic acid content in *Garcinia* species butter

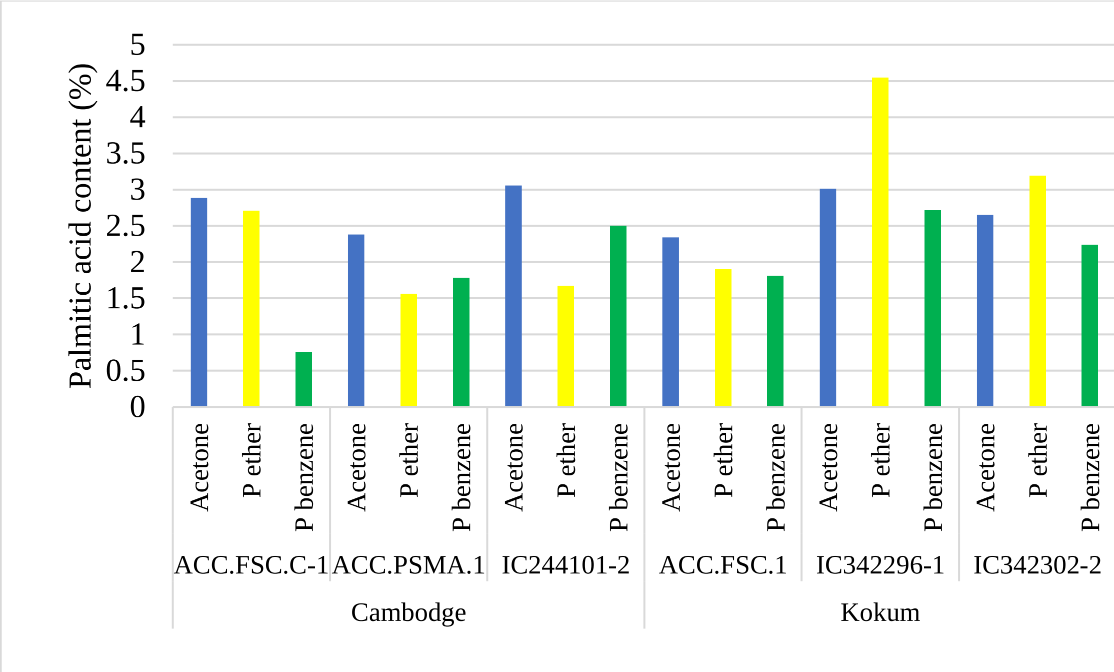


Fig. 4.8. Variation of palmitic acid content in *Garcinia* species butter

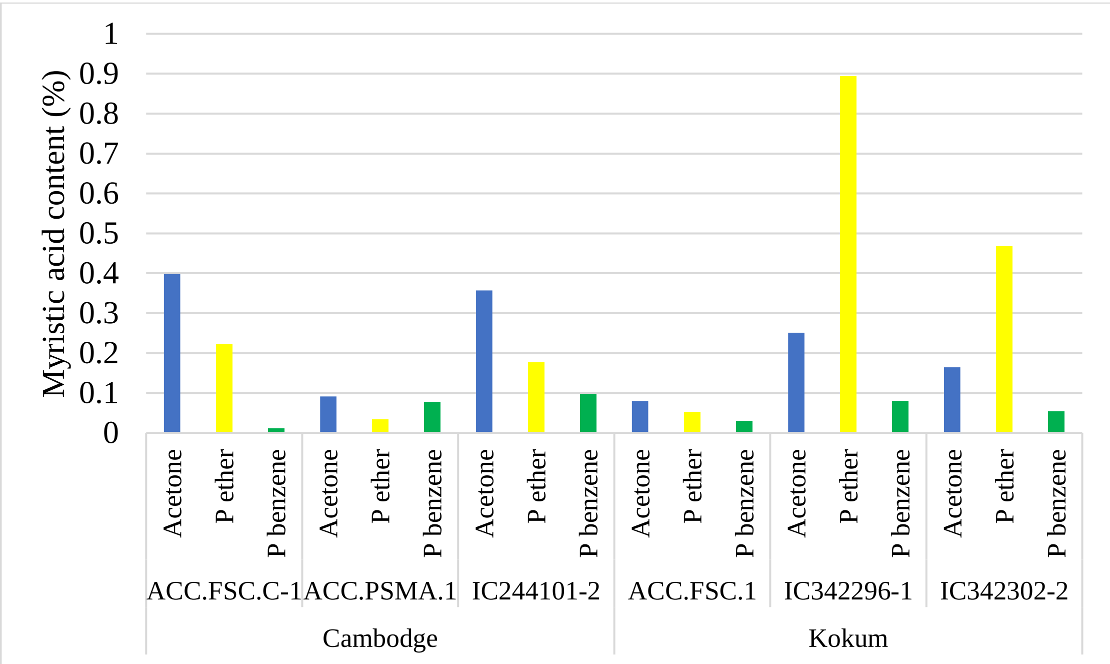


Fig.4.9. Variation of myristic acid content in *Garcinia* species butter

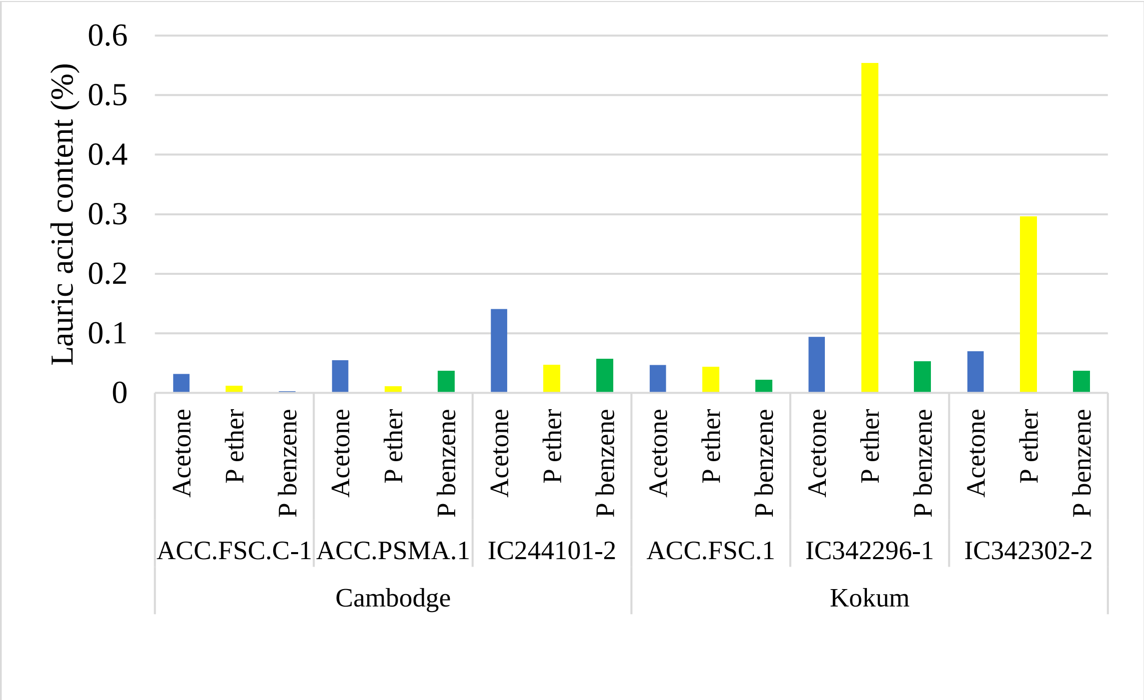


Fig. 4.10. Variation of lauric acid content in *Garcinia* species butter

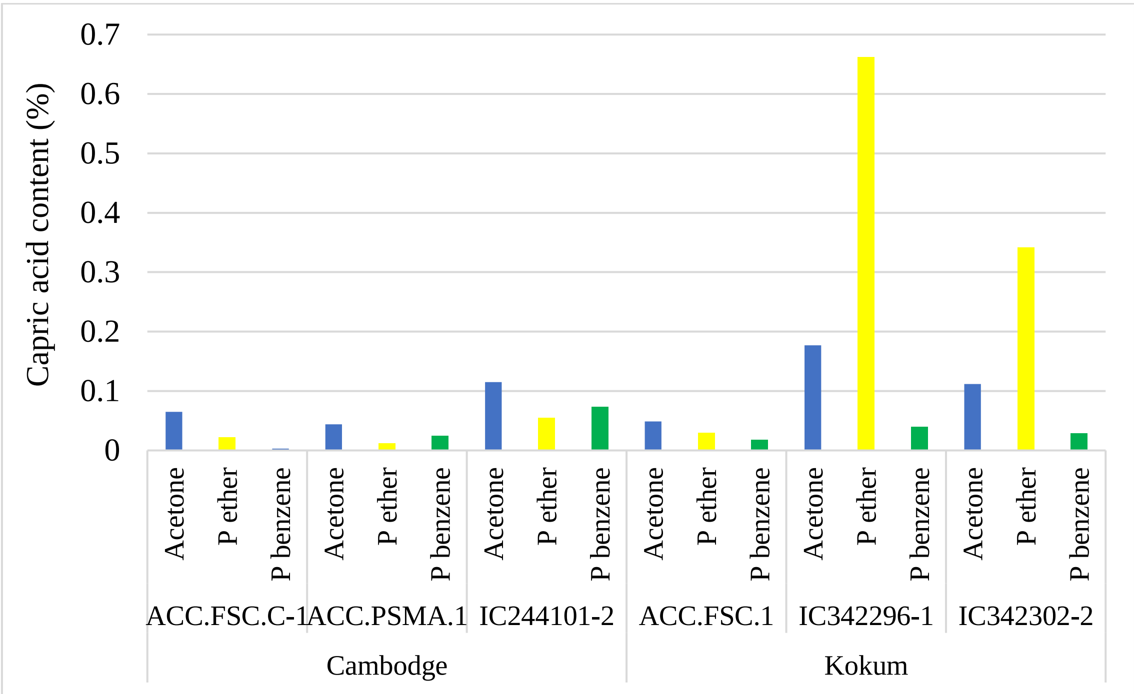


Fig. 4.11. Variation of capric acid content in *Garcinia* species butter

Milk butter

Kokum butter

Cambodge butter

Cocoa butter

Plate 4.6. Organoleptic evaluation of different butters

For texture of different butters, milk butter (3.63) attained the highest mean rank score followed by cocoa butter (2.30), kokum butter (2.23) and cambodge butter (1.83), whereas the mean score ranged from 7.29 to 8.69. Milk butter observed the highest mean score (8.69), followed by cocoa butter (7.64), kokum butter (7.47) and cambodge butter (7.29).

For taste of different butters, the mean score and mean rank score varied 7.47 (kokum butter) to 8.80 (milk butter) and 1.67 (kokum butter) to 3.77 (milk butter). The highest mean score was obtained in milk butter (8.80) followed by cocoa butter (8.04), cambodge butter (7.53) and kokum butter (7.29).

For different butters, the mean score for after taste varied from 7.15 to 8.64 and mean rank score ranged from 1.57 to 3.73. Milk butter (8.64) obtained the highest mean score followed by cocoa butter (7.98), kokum butter (7.55) and cambodge butter (7.15). The mean rank score was observed higher in milk butter (3.73) followed by cocoa butter (2.60), kokum butter (2.10) and cambodge (1.57).

The mean score for overall acceptability of different butters varied from 7.04 to 8.71 whereas mean rank score varied from 1.63 to 3.63. The highest mean score and mean rank score for overall acceptability was observed in milk butter (8.71 & 3.63, respectively) followed by cocoa butter (8.26 & 3.00 respectively), cambodge butter (7.15 & 1.73, respectively) and kokum butter (7.04 & 1.63, respectively).

4.4.2. Organoleptic qualities of burfi prepared using different butters

Mean score and mean rank score for various quality attributes of burfi prepared using different butters are given in Table 4.14.

The mean score and mean rank score for appearance of burfi prepared using different butters ranged from 6.95 (kokum burfi) to 8.05 (cambodge burfi) and 2.26 (kokum burfi) to 3.34 (milk burfi). The highest mean score for appearance was obtained in cambodge burfi (8.05) followed by milk burfi (7.89), cocoa burfi (7.68) and kokum burfi (6.95).

For different burfi, the mean score for colour varied from 6.95 to 8.11 and mean rank score ranged from 1.68 to 3.73. Milk butter (8.64) obtained the highest mean score followed by cocoa butter (7.98), kokum butter (7.55) and cambodge butter (7.15). The mean rank score was observed higher in milk butter (3.73) followed by cocoa butter (2.60), kokum butter (2.10) and cambodge (1.57).

For flavour of burfi, the mean score ranged from 6.74 (cocoa burfi) to 8.26 (milk burfi) whereas mean rank score ranged from 1.66 (cocoa burfi) to 3.13 (milk burfi). Milk burfi recorded the higher mean score (8.26) followed by cambodge burfi (8.00), kokum burfi (7.68) and cocoa burfi (6.74).

For texture of burfi, the highest mean score and mean rank score was attained in milk burfi (8.31 & 3.13, respectively) which was followed by cambodge burfi (8.31 & 2.68, respectively), kokum burfi (8.00 & 2.13, respectively) and cocoa burfi (7.42 & 2.05, respectively).

For burfi prepared with different kinds of butter, the mean score and mean rank score for taste varied from 6.37 (cocoa burfi) to 8.37 (milk burfi) and 1.37 (cocoa burfi) to 3.16 (milk burfi), respectively. The mean score was obtained higher in milk burfi (8.37) followed by cambodge burfi (7.95), kokum burfi (7.89) and cocoa burfi (6.37).

For after taste of burfi, the highest mean score and mean rank score was attained in milk burfi (8.10 & 3.26, respectively) which was followed by kokum burfi (7.68 & 2.71, respectively), cambodge burfi (7.42 & 2.58, respectively) and cocoa burfi (6.00 & 1.45, respectively).

The mean score and mean rank score for overall acceptability varied from 6.58 to 8.31 and 1.39 to 3.26. Milk burfi (8.31) obtained the highest mean score followed by cambodge burfi (7.95), kokum burfi (7.84) and cocoa burfi (6.58). The mean rank score was observed higher milk burfi (3.26), cambodge burfi (2.76), kokum burfi (2.58) and cocoa burfi (1.39).

4.4.3. Organoleptic qualities of cake prepared using different butters

Mean score and mean rank score for various quality attributes of cake prepared using different butters are given in Table 4.15.

For appearance, the mean score varied from 7.31 to 8.31 whereas mean rank score of 1.95 to 3.16. Milk butter cake (8.31) was obtained the highest mean score followed by

T_1- Burfi of milk butter

T_2- Burfi of kokum butter

T_3- Burfi of cambodge butter

T_4- Burfi of cocoa butter

Plate 4.7. Burfi prepared using different butters

T_1- Cake of milk butter

T_2- Cake of kokum butter

T_3- Cake of cambodge butter

T_4- Cake of cocoa butter

Plate 4.8. Cake prepared using different butters

Plate 4.9. Organoleptic evaluation of burfi

Plate 4.10. Organoleptic evaluation of cake

kokum cake (7.84), cambodge cake (7.74) and cocoa cake (7.31). The mean rank score was obtained higher in milk butter cake (3.16) followed by kokum cake (2.47), cambodge cake (2.42) and cocoa cake (1.95).

For cake prepared with different butters, the mean score and mean rank score for colour were observed high in milk butter cake (8.37 & 3.18, respectively) followed by kokum cake (7.84 & 2.63, respectively), cambodge cake (7.68 & 2.34, respectively) and cocoa cake (7.05 & 1.84, respectively).

For flavour of different cakes, the mean score and mean rank score were observed higher in milk butter cake (8.10 & 3.13, respectively) followed by kokum cake (7.89 & 2.95, respectively), cambodge cake (7.53 & 2.34, respectively) and cocoa cake (6.16 & 1.58, respectively).

For cake prepared with different butters, the mean score and mean rank score for texture were observed higher in milk butter cake (8.21 & 3.34, respectively) followed by cambodge cake (7.58 & 2.55, respectively), kokum cake (7.27 & 2.26, respectively) and cocoa cake (6.79 & 1.84, respectively).

For taste, the mean score and mean rank score were obtained higher in milk butter cake (8.10 & 3.13, respectively) followed by kokum cake (7.95 & 2.87, respectively), cambodge cake (7.68 & 2.61, respectively) and cocoa cake (6.10 & 1.39, respectively).

For quality attribute like after taste, the mean score and mean rank score were ranged from 5.89 (cocoa cake) to 7.95 (milk butter cake) and 1.39 (cocoa cake) to 3.11 (kokum cake), respectively. The mean score was higher in milk butter cake (7.95) followed by kokum cake (7.79), cambodge cake (7.37) and cocoa cake (5.89). Whereas mean rank score was obtained higher in kokum cake (3.11) followed by milk butter cake (3.08), cambodge cake (2.42) and cocoa cake (1.39).

For overall acceptability of cake, milk butter cake (3.11) had the highest mean rank score followed by kokum cake (3.05), cambodge cake (2.37) and cocoa cake (1.47). Whereas for mean score, milk butter cake (8.16) was recorded higher, followed by kokum cake (7.95), cambodge cake (7.53) and cocoa cake (6.53).

Table 4.13. Organoleptic qualities for different types of butter

Treatments	Appearance	Colour	Flavour	Texture	Taste	After Taste	Overall acceptability	Total score
Milk butter	8.64 (3.90)	8.49 (3.63)	8.73 (3.72)	8.69 (3.63)	8.80 (3.77)	8.64 (3.73)	8.71 (3.63)	60.7
Kokum butter	7.69 (2.43)	7.58 (2.50)	7.16 (1.81)	7.47 (2.23)	7.47 (1.67)	7.55 (2.10)	7.04 (1.63)	51.96
Cambodge butter	7.53 (2.27)	7.82 (2.60)	6.89 (1.44)	7.29 (1.83)	7.53 (1.90)	7.15 (1.57)	7.15 (1.73)	51.36
Cocoa butter	6.82 (1.40)	6.64 (1.27)	8.10 (3.03)	7.64 (2.30)	8.04 (2.67)	7.98 (2.60)	8.26 (3.00)	53.48
Kendall's W	0.723^{**}	0.626^{**}	0.759^{**}	0.438^{**}	0.615^{**}	0.569^{**}	0.625^{**}	

Values in parentheses are mean rank score based on Kendall's W

**-Significant at 1% level

Table 4.14. Organoleptic qualities of burfi

Treatments	Appearance	Colour	Flavour	Texture	Taste	After Taste	Overall acceptability	Total score
T_1	7.89 (3.34)	8.10 (2.95)	8.26 (3.13)	8.31 (3.13)	8.37 (3.16)	8.10 (3.26)	8.31 (3.26)	57.34
T_2	6.95 (2.26)	6.95 (1.68)	7.68 (2.45)	7.47 (2.13)	7.89 (2.76)	7.68 (2.71)	7.84 (2.58)	52.46
T_3	8.05 (2.55)	8.11 (3.03)	8.00 (2.76)	8.00 (2.68)	7.95 (2.71)	7.42 (2.58)	7.95 (2.76)	55.48
T_4	7.68 (1.84)	7.68 (2.34)	6.74 (1.66)	7.42 (2.05)	6.37 (1.37)	6.00 (1.45)	6.58 (1.39)	48.47
Kendall's W	0.306^{**}	0.277^{**}	0.320^{**}	0.209^{**}	0.418^{**}	0.406^{**}	0.438^{**}	

Values in parentheses are mean rank score based on Kendall's W

**-Significant at 1% level

T_1- Burfi prepared using milk butter

T_2- Burfi prepared using kokum butter

T_3- Burfi prepared using cambodge butter

T_4- Burfi prepared using cocoa butter

Table 4.15. Organoleptic qualities of cake

Treatments	Appearance	Colour	Flavour	Texture	Taste	After Taste	Overall acceptability	Total score
T_1	8.31 (3.16)	8.37 (3.18)	8.10 (3.13)	8.21 (3.34)	8.10 (3.13)	7.95 (3.08)	8.16 (3.11)	57.2
T_2	7.84 (2.47)	7.84 (2.63)	7.89 (2.95)	7.27 (2.26)	7.95 (2.87)	7.79 (3.11)	7.95 (3.05)	54.53
T_3	7.74 (2.42)	7.68 (2.34)	7.53 (2.34)	7.58 (2.55)	7.68 (2.61)	7.37 (2.42)	7.53 (2.37)	53.11
T_4	7.31 (1.95)	7.05 (1.84)	6.16 (1.58)	6.79 (1.84)	6.10 (1.39)	5.89 (1.39)	6.53 (1.47)	45.83
Kendall's W	0.179^{**}	0.249^{**}	0.361^{**}	0.306^{**}	0.415^{**}	0.506^{**}	0.396^{**}	

Values in parentheses are mean rank score based on Kendall's W

**-Significant at 1% level

T_1- Cake prepared using milk butter

T_2- Cake prepared using kokum butter

T_3- Cake prepared using cambodge butter

T_4- Cake prepared using cocoa butter

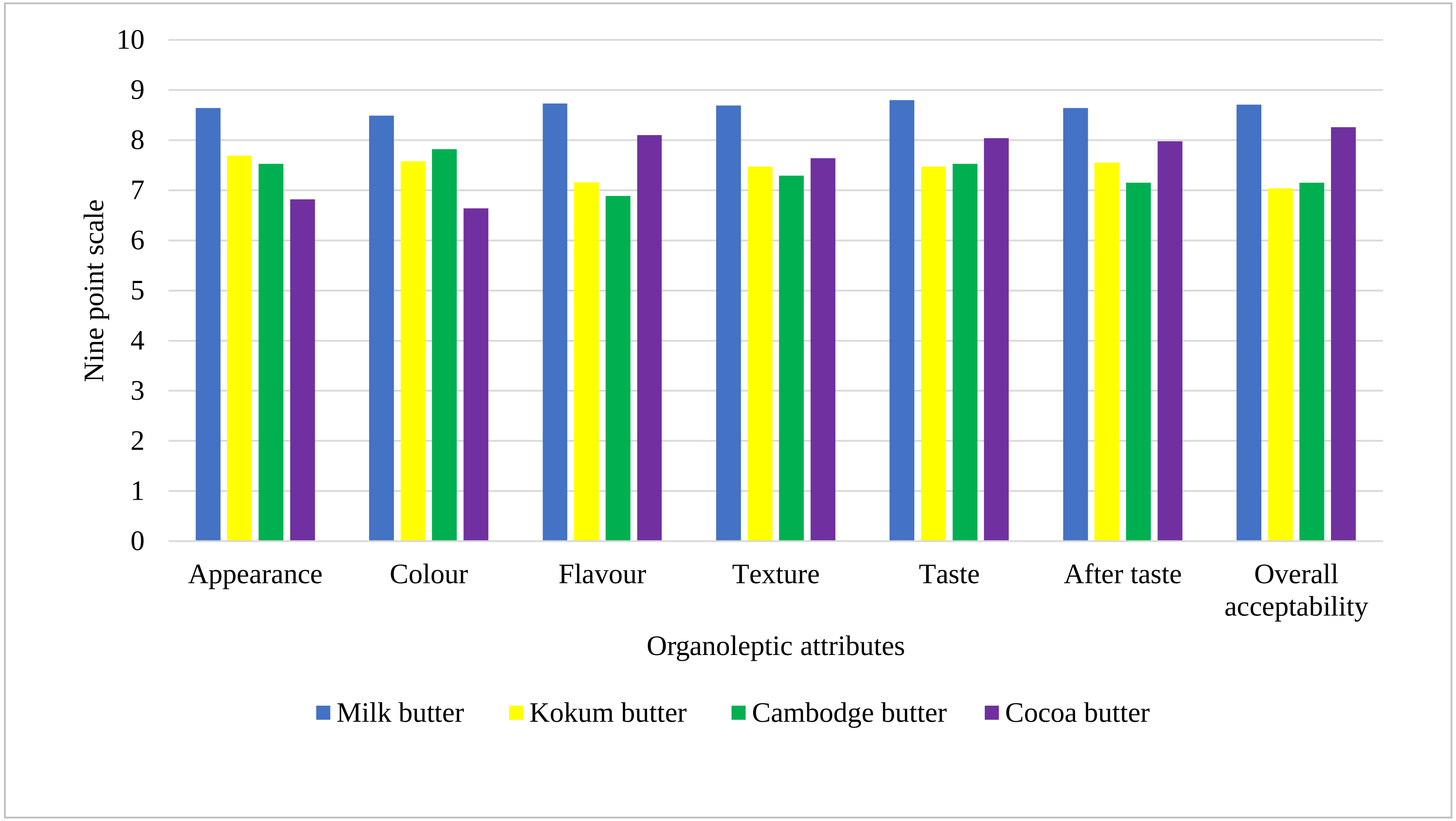


Fig. 4.12. Mean score card for different types of butter

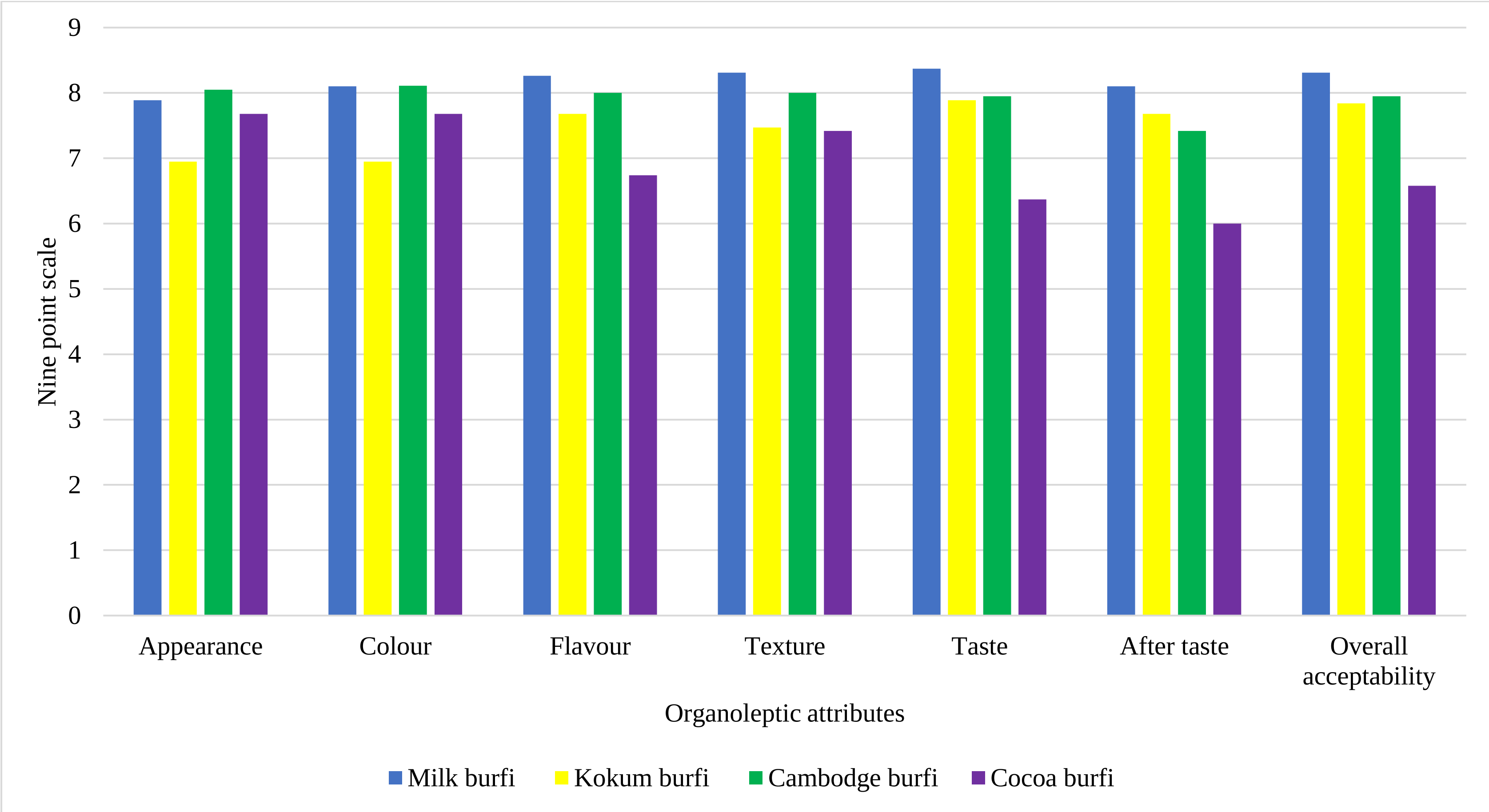


Fig. 4.13. Mean score card for burfi prepared using different butters

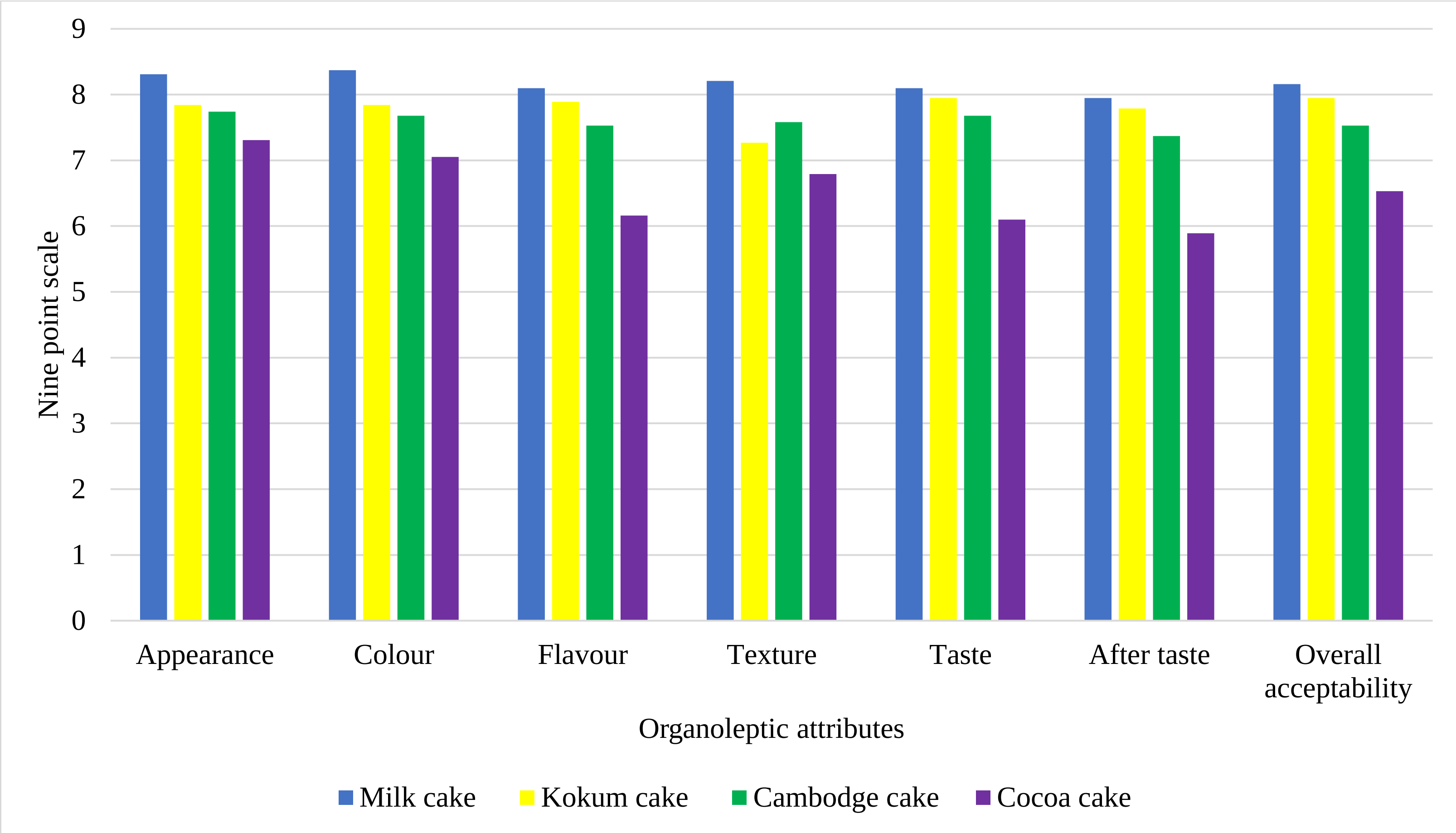


Fig. 4.14. Mean score card for cake prepared using different butters

DISCUSSION

5. DISCUSSION

A study was conducted to characterize the physico-chemical and nutritional properties of seed and seed butter of cambodge and kokum under the title ‘‘Physico-chemical and nutritional analysis of seed and seed-butter of *Garcinia* spp.”. The characters on variability in biochemical and nutritional parameters of seeds including extraction of butter. Further physico-chemical, nutritional properties and fatty acids profiling of butter as well as organoleptic evaluation of butter and their products were assessed. The results of the present study are discussed in this chapter.

5.1. BIOCHEMICAL AND NUTRITIONAL QUALITIES OF SEEDS

5.1.1. Biochemical analysis of seeds

5.1.1.1. Moisture

The level of moisture content in seed has a significant impact on fungal colonization throughout the pre-harvest, post-harvest and storage period. The seed moisture content is known to influence variations in the chemical makeup of the seeds. The most powerful factor influencing seed longevity is the moisture level during storage. In the present study, the moisture content of seed powder ranged from 4.56 to 7.42 per cent and 10.49 to 12.50 per cent in cambodge and kokum, respectively. A low coefficient of variation for moisture content in cambodge and kokum was 0.84 and 1.60 per cent, respectively.

Previously Manohar *et al*. (2014) reported the moisture content of seeds in *Garcinia xanthochymus* (11.96 %). In another study, Ajayi *et al*. (2007) reported the moisture content of *Garcinia mangostana* seeds (13.08 %). According to Chate *et al*. (2019), the moisture content of kokum seeds was 10.20 per cent. In this study cambodge seed powder recorded low moisture content, whereas kokum seed powder had high moisture content. Kokum moisture content can be comparable with the previous studies. Cambodge seed moisture was recorded lower than the kokum which implies the longevity of seeds during storage.

5.1.1.2. Starch

Starch is an important source of nutrition in daily diet. Starch is generated in growing seeds, where it serves as an energy source for seedling establishment. In the present study, starch content of cambodge and kokum seed powder varied from 0.448 to 0.672 g per 100 g and 0.478 to 0.662 g per 100 g, respectively. Coefficient of variation for starch content in cambodge and kokum was 2.18 (%) and 2.86 (%), respectively.

According to Noor *et al*. (2016), a higher amount of starch was recorded in *Garcinia mangostana*. The other species of *Garcinia viz. G. hombroniana, G. prainiana* and *G. atroviridis* had shown small amount of starch content in seeds. It means that recalcitrant seeds may use starch in lipid production, as lipids are chosen as storage reserves. Murmu *et al*. (2016) reported the starch content in raw fruit of *Garcinia xanthochymous* was 9.06 per cent. In the present study, lower amount of starch content was observed in both cambodge and kokum seed powder. From the previous study, it was observed that starch content was higher in fruits and it is lower in *Garcinia* species seeds.

5.1.1.3. Total carbohydrates

In the present investigation, total carbohydrates content in cambodge and kokum seed powder ranged from 11.03 to 14.93 g per 100 g and 4.03 to 5.13 g per 100 g, respectively. Significantly highest total carbohydrate content was recorded in cambodge accessions. A low coefficient of variation was observed in cambodge (1.93 %) and kokum (3.73 %).

Parthasarathy and Nandakishore (2014) reported the total carbohydrate content in fruits of *Garcinia gummi-gutta* (7.11 g per 100 g) and *Garcinia indica* (6.24 g per 100 g). According to Chate *et al*. (2019), carbohydrate content in kokum was 29.50 per cent. In this study, cambodge seed powder recorded high total carbohydrates content than that of kokum seed powder. The carbohydrate content of cambodge was higher in seeds than fruits. In case of kokum, the carbohydrate content was higher in fruits than seeds. From the previous study, kokum was observed more carbohydrate content than present study. This could be due to genotypic and environmental variation.

5.1.1.4. Protein

The protein content of cambodge and kokum seed powder varied from 1.17 to 1.39 per cent and 0.18 to 0.66 per cent, respectively. From the present study, significantly highest protein content was recorded in cambodge seed powder than that of kokum seed powder.

Parthasarathy and Nandakishore (2014) reported the protein content of fruits in *Garcinia gummi-gutta* (3.25 g per 100g) and *Garcinia indica* (4.78 g per 100g). Chate *et al*. (2019) reported the protein content of kokum seed flour was 9.10 per cent. It was observed that protein content was higher in fruits when compared to the seeds of both cambodge and kokum. In the previous study, protein content of kokum seed flour was high when compared to present study. This could be due to genotypic variation, environmental influence and moisture content of seeds.

5.1.1.5. Total fat

Fats are a kind of energy storage found in plants, particularly in seeds. Fats are the second most abundant source of energy for living cells. A high quantity of total fat was recorded in the seed powder of both kokum and cambodge. In the present study, significantly higher amount of total fat was obtained in kokum (40.28 %) than that of cambodge seed powder (38.07 %). Low coefficient of variation was obtained in cambodge and kokum.

Parthasarathy *et al.* (2014) reported the fat content in cambodge (46.5 %) and kokum seeds (29.9 %). According to Raveena (2019), the fat content in kokum seeds ranged from 25.65 to 35.69 per cent. In the present study, fat content was recorded higher in kokum when compared to previous study. The difference in fat content is may be due to genetic variation and size of the seed kernels.

5.1.1.6. Total ash or Total minerals

In the present investigation, total ash content in cambodge and kokum seed powder ranged from 2.26 to 2.77 per cent and 2.30 to 2.42 per cent, respectively. Cambodge seed powder recorded significantly higher ash content than that of kokum seed powder

According to Ajayi *et al*. (2007), the ash content in seeds of *Garcinia mangostana* was 1.99 g per 100 g. Ebana *et al*. (2017) reported the ash content in *Garcinia kola* seeds (3.50 %). In another study, Chate *et al*. (2019) reported the ash content of full flat flour in kokum (2.90 %). The ash content of both cambodge and kokum seed powder can be comparable with the previous studies.

5.1.1.7. Dietary fibre

Dietary fibre adds bulk to the diet and aids digestion, prevents constipation and helps control of body weight. Coefficient of variation was low for dietary fibre in cambodge (1.49 %) and kokum (1.17 %). The dietary fibre of cambodge and kokum seed powder ranged from 1.45 to 1.74 per cent and 4.50 to 4.64 per cent, respectively. Higher amount of dietary fibre was recorded in kokum when compared to cambodge.

Ikeda *et al*. (2021) reported the dietary fibre in full ripe seeds of *Garcinia humilus* (28.20 g per 100 g). In present study, the dietary fibre recorded in kokum and cambodge seeds was lower than previous study. This could be due to species variation and genetic factors.

5.1.2. Nutritional composition of seeds

5.1.2.1. Calcium

Calcium is essential for the formation and maintenance of strong bones (Pravina *et al*., 2013). Calcium is thought to be an important factor governing fruit storage quality in many fruit tissues (Lechaudel *et al*., 2005). In the present investigation, calcium content of seed powder in cambodge and kokum varied from 147.80 to 176.70 mg per 100 g and 45.90 to 50.50 mg per 100 g, respectively. Significantly higher calcium content was recorded in cambodge when compared with kokum. The coefficient of variation obtained for calcium content in cambodge and kokum accessions was 4.51 and 3.61 per cent, respectively.

According to Raysad (2016), calcium content in fruits of *Garcinia gummi-gutta* ranged from 105.44 to 212.25 mg per 100 g. Whereas Raveena (2019) reported calcium content in *Garcinia indica* fruits (4.56 to 7.45 mg per 100 g). According to Chate *et al*. (2019), the calcium composition of kokum seed flour was 24.10 mg per 100 g. In kokum, calcium content was higher in seeds when compared to fruits. Whereas in cambodge, calcium content of fruits and seeds had comparable values. In the present study, kokum seed powder recorded high calcium level compared to previous study. This could be due environmental influence on the trees grown or genetic factors.

5.1.2.2. Iron

Iron is a mineral that is essential for the production of blood. It is a component of hemoglobin, a protein that transports oxygen from our lungs throughout the body. It aids in the storage and utilization of oxygen by our muscles. The iron content of cambodge and kokum ranged from 15.30 to 16.80 mg per 100 g and 13.90 to 14.70 mg per 100 g, respectively. A high coefficient of variation was recorded for iron content in both cambodge and kokum.

Chate *et al*. (2019) reported iron content in kokum seed flour (8.80 mg per 100 g). In another study, Joseph *et al*. (2017) reported the iron content of mangosteen seeds (34.00 mg per 100 g). In the present study, iron content of kokum seed powder was higher than the previous study. This could be due environmental influence on the trees grown or genetic factors.

5.1.2.3. Potassium

Potassium is an important mineral that involves in metabolism and proper functioning of various tissues and organs in the human body (Ozcan, 2004). The potassium content of cambodge seed powder ranged from 442.00 to 514.60 mg per 100 g. Whereas potassium content in kokum seed powder ranged from 568.00 to 652.70 mg per 100 g.

Raysad (2016) reported potassium content in fruits of cambodge ranged from 115.80 to 227.10 mg per 100 g. In another study, Raveena (2019) reported the potassium content in fruits of kokum ranged from 25.16 to 33.05 mg per 100 g. Joseph *et al*. (2017) reported the potassium content of mangosteen seeds (1036.00 mg per 100 g). In the present, the potassium content of seeds was recorded as higher than fruits in both cambodge and kokum.

5.1.2.4. Sodium

Sodium is the primary element that regulates acid-base balance and is involved in the maintenance of body fluid osmotic pressure (Soetan *et al*., 2010). In the present study, the sodium content of kokum and cambodge seed powder varied from 29.10 to 35.30 mg per 100 g and 14.00 to 16.60 mg per 100 g, respectively.

Chate *et al*. (2019) reported the sodium content in kokum seed flour (34.80 mg per 100 g). Raysad (2016) reported the sodium content in fruits of cambodge ranged from 41.00 to 98.00 mg per 100 g. In another study, Raveena (2019) reported the sodium content in kokum fruits ranged from 0.23 to 1.92 mg per 100 g. Joseph *et al*. (2017) reported sodium content of mangosteen seeds (840.00 mg per 100 g). In the present study, it was observed that sodium content of seeds was recorded as higher than fruits in kokum and vice-versa in cambodge.

5.2. EXTRACTION, PHYSICO-CHEMICAL AND NUTRITIONAL QUALITIES OF BUTTER

5.2.1. Extraction of butter

The quantity of butter extracted in the study was significantly higher in kokum (49.61 %) when compared to cambodge (44.98 %) using acetone as solvent. In case of butter extracted using petroleum ether and petroleum benzene, the quantity of butter was recorded higher in kokum (40.29, 42.25 %, respectively) than that of cambodge (38.41 %, 37.25 %, respectively). The butter extracted through acetone was light brown in cambodge and kokum. Whereas white colour of butter was extracted using petroleum ether and petroleum benzene. Butter extracted using petroleum ether is most accepted for the daily use. Though butter yield from petroleum ether was white in colour, the extracted butter would be refined with alkali and deodorized for increasing the edible nature. The results of the present study show cambodge butter yield ranged from 43.26 to 47.19 percent in acetone, 36.30 to 42.01 per cent in petroleum ether, 33.86 to 41.32 per cent in petroleum benzene. In case of kokum, butter yield was ranged from 48.88 to 50.67 per cent in acetone, 39.27 to 41.57 per cent in petroleum ether, 41.96 to 42.67 per cent in petroleum benzene. In the previous study Nagavekar *et al*. (2019), reported butter recovery from kokum (42.80 %). Butter yield was recorded higher in kokum seeds when compared to cambodge seeds.

5.2.2. Physico-chemical analysis of butter

5.2.2.1. Physical state at room temperature

Both cambodge and kokum butter are solid at room temperature which makes them ideal for preparation of chocolates and easy to handle during cooking. This was in accordance with the work reported by Raveena (2019) and Raysad (2016). Whereas cocoa butter is solid at room temperature, it would meet with the human body temperature. It is in contrast both cambodge and kokum butter would not completely melt at human body temperature.

5.2.2.2. Oil or Total fat

The total fat content in seed butter of cambodge and kokum varied from 99.49 to 99.81 per cent and 99.81 to 99.89 per cent, respectively. The fat content of milk butter was 80.83 per cent lower than both cambodge and kokum butter (FSSAI, 2017). This is due to presence of curd in milk butter which is absent in *Garcinia* species. The fat content in milk butter was lower than that of cambodge and kokum butter as per FSSAI (2017) report.

5.2.2.3. Moisture content

The quality and longevity of the butter was influenced by moisture content. The moisture content of seed butter varies from 0.19 to 0.51 per cent and 0.11 to 0.20 per cent in cambodge and kokum, respectively. Ramachandran (2014) reported the moisture content of kokum butter (0.50 %). In another study, O-Ebongue *et al*. (2013) found the moisture content of crude palm oil was 0.20 per cent. Kokum seed was recorded lower moisture content than cambodge seed butter. It is observed that increase in moisture content could accelerates the oxidation and there by loses the quality.

5.2.2.4. Refractive index

In the present study, the refractive index of cambodge (1.459) and kokum butter (1.457) was recorded with comparable values. Previously Manohar *et al*. (2014) reported the refractive index of *Garcinia xanthochymus* seed oil (1.488). In another study, Ajayi *et al*. (2007) reported refractive index of *Garcinia mangostana* seed oil (1.482). The refractive index of cow butter ranged from 1.44 to 1.50 (Agamy, 2006). From present study, refractive index of *Garcinia* butter was higher when compared to milk butter. The low refractive index indicates the good quality of butter and less spoilage due to oxidation. The higher refractive index value of cambodge and kokum butter could be due to higher proportion of long chain fatty acids (C14-C18).

5.2.2.5. Melting point and boiling point

The melting point of cambodge ranged from 38.46 ℃ to 39.62 ℃. Whereas in case of kokum the melting point was 36.92 ℃ to 39.08 ℃. Among the *Garcinia* species studied, melting point was significantly highest in cambodge butter than that of kokum butter. Raysad (2016) reported melting point of cambodge butter was 36-42 ℃. Raveena (2019) reported melting point of kokum butter (35-44 ℃). According to Parthasarathy *et al*. (2014), the melting point of *Garcinia gummi-gutta* was 39.4 ℃ and in *Garcinia indica* was 40.3 ℃. Cocoa butter melting point was reported as 27- 40 ℃ (Kalse et al., 2021). Because of this melting point behaviour, cocoa butter would melt easily with the human body temperature or in the higher atmosphere temperature. To avoid melting of cocoa butter, cambodge and kokum butter can be blended. More concentration of long chain fatty acids (C14-C18) than medium chain fatty acids (C10-C12) could be the reason for high melting point of butter. In the present study, boiling point of both cambodge and kokum butter was greater than 300 ℃. According to Nag *et al*. (1995), boiling point of *Putranjiva roxburghii* oil was 255-261 ℃. The presence of high boiling point in cambodge and kokum butter indicates the purity and deep frying nature.

5.2.2.6. Solubility

Kokum and cambodge butter were more soluble in chloroform compared to alcohol and water. According to Vidhate and Singhal (2013), kokum butter was more soluble in t-butanol compared to ethanol and iso-propanol. The insolubility of butter is due to the presence of fats in butter.

5.2.2.7. pH

pH of food plays an important role in food processing industry. In present study, the pH of seed butter varied from 4.97 to 5.69 and 4.9 to 5.51 in cambodge and kokum with low coefficient of variation of 0.81 and 0.61 per cent, respectively. According to Adewumi *et al*. (2017), milk butter pH was 7.01 and cocoa butter pH ranged from 5.2 to 6.0. Previous report shows that milk butter has more pH, whereas cocoa butter pH range was comparable with cambodge and kokum butter.

5.2.2.8. Ash content or Total minerals

In the present study, ash content of seed butter in cambodge and kokum ranged from 0.133 to 0.144 per cent and 0.114 to 0.118 per cent, respectively. Chukwu and Adgidzi (2008) reported the ash content in shea butter (1.26 %) and groundnut oil (1.8-3.1 %). *Garcinia* species had shown less ash content when compared to shea butter and groundnut oil.

5.2.2.9. Acid value

Acid value is a measure of fats susceptibility to decomposition and represents both their freshness and storage quality (Akbar *et al*., 2009). There was not much variation observed for acid value in kokum and cambodge accessions. The acid value of cambodge and kokum had a CV of 9.75 per cent and 5.60 per cent, respectively. The acid value of cambodge and kokum butter ranged from 5.04 to 5.98 mg KOH per g and 4.25 to 6.83 mg KOH per g, respectively.

This indicates that cambodge and kokum butter are good for consumption. According to Raysad (2016), acid value of cambodge butter ranged from 3.09 to 4.49 mg KOH per g. Raveena (2019), reported the acid value of kokum butter (2.60 to 3.40 mg KOH per g). In another study, Parthasarathy *et al*. (2014) recorded the acid value of kokum (4.90 mg NaOH per g) and cambodge butter (3.70 mg NaOH per g). According to Choppa *et al*. (2015) the acid value in cambodge was 5.04 mg KOH per g. In the present study, both the species has shown higher acid values when compared to previous study. This may be due to inheritable characters of accessions or environmental influence on the trees grown.

5.2.2.10. Saponification value

The saponification value of a fat indicates the nature of the fatty acid present. Presence of high saponification value indicates the presence of lower fatty acids with low molecular weight. High saponification fats are very useful in the production of shampoo and liquid soap (Akbar *et al.*, 2009). In the present study, the saponification value of kokum butter and cambodge butter varied from 174.81 to 186.10 mg KOH per g and 185.53 to 190.62 mg KOH per g, respectively. The coefficient of variation obtained for saponification value in kokum and cambodge was low.

Parthasarathy *et al*. (2014) reported the saponification value of cambodge (187.90 mg KOH per g) and kokum butter (200.20 mg KOH per g). Raveena (2019) reported the saponification value of kokum butter varied from 171.10 to 194.94 mg KOH per g. According to Raysad (2016), saponification value of cambodge butter ranged from 171.81 to 189.34 mg KOH per g. In the present study both cambodge and kokum had high saponification value, hence these can be used in preparation of soaps and shampoos.

5.2.2.11. Iodine value

The iodine value of fat is used to determine its unsaturation. This value can be used to predict the rancidity of fat (Akbar *et al*., 2009). The iodine value of cambodge butter and kokum butter ranged from 53.63 to 62.44 g per 100 g and 37.54 to 38.62 g per 100 g, respectively.

Raysad (2016) reported iodine value of cambodge butter varied from 48.22 to 76.14 g per 100 g. Raveena (2019) reported iodine value of kokum butter ranged from 36.41 to 40.56 g per 100 g. According to Parthasarathy *et al*. (2014), the iodine value of cambodge butter and kokum butter was 50.20 g per 100 g and 39.40 g per 100 g. The iodine value of cocoa butter was 32.00 to 35.00 g per 100 g (Kalse *et al*. 2021). In this study cambodge seed butter recorded high iodine value, whereas kokum seed butter had low iodine value. The iodine value of kokum butter was near to the cocoa butter. Hence kokum butter could be good source of fat in daily life.

5.2.2.12. Peroxide value

In present study, the peroxide value of cambodge and kokum butter ranged from 4.34 to 4.41 meq per kg and 5.77 to 5.84 meq per kg, respectively. Ajayi *et al*. (2007) reported the peroxide value of mangosteen butter (3.27 meq per kg). According to Kalse *et al*. (2021), the peroxide value of cocoa butter was 1.00 to 1.10 meq per kg. The lower peroxide value indicates the composition of good oil and more shelf life of oil. Peroxide values of cambodge butter were lower than that of kokum butter. Both kokum and cambodge butter recorded more peroxide value than cocoa butter.

5.2.2.13. Ester value

The ester value varied from 180.49 to 184.31 mg KOH per g and 170.76 to 178.99 mg KOH per g for cambodge and kokum, respectively. A low coefficient of variation was obtained in cambodge (1.54 %) and kokum (1.34 %). Mohammed *et al*. (2017) reported the ester value kokum butter (157.90 mg KOH per g). Hesham *et al*. (2015) reported the ester value in different oils *viz.* olive oil (142.50 mg KOH per g), melon oil (142.40 mg KOH per g), sunflower oil (124.00 mg KOH per g) and virgin coconut oil (254.60 mg KOH per g), marula traditional oil (308.16 mg KOH per g). The ester value of both cambodge and kokum butter was higher than olive oil, sunflower oil and melon oil.

5.2.3. Nutritional analysis of butter

5.2.3.1. Calcium

The calcium content in seed butter of different accessions of cambodge and kokum ranged from 20.40 to 25.20 mg per 100g and 16.00 to 23.90 mg per 100 g, respectively. The coefficient of variation for calcium content in seed butter of cambodge and kokum was 6.26 (%) and 6.47 (%), respectively. According to FSSAI (2017) reports, the amount of calcium content recorded in milk butter was 18.00 mg per 100 g. From the previous study, it can be compared that range of cambodge and kokum butter are comparable with milk butter.

5.2.3.2. Iron

In the present investigation, the iron content of seed butter in cambodge and kokum ranged from 11.40 to 15.10 mg per 100 g to 10.10 to 15.60 mg per 100 g, respectively. A trace amount of iron content was recorded in milk butter (FSSAI, 2017). Galvao *et al*. (1976) reported the iron composition of peanut butter was 1.62 mg per 100 g. In the present study, both the species had shown higher iron content when compared to previous study. *Garcinia* butter is rich in iron content and may be used to treat anemia.

5.2.3.3. Potassium

In the present study, potassium content showed low coefficient of variation in cambodge (1.59 %) and kokum (1.66 %). Potassium content in cambodge and kokum seed butter varied from 31.30 to 35.50 mg per 100 g and 17.90 to 18.70 mg per 100 g, respectively. According to FSSAI (2017), potassium content in milk butter was 27.00 mg per 100 g. In the present study observed higher amount of potassium in cambodge butter when compared to milk butter.

5.2.3.4. Sodium

The sodium content of seed butter of cambodge and kokum varied from 14.30 to 15.60 mg per 100 g and 17.30 to 18.10 mg per 100 g, respectively. A low coefficient of variation was obtained for sodium content in cambodge (2.54 %) and kokum (4.90 %). The sodium content in milk butter was 9.00 mg per 100 g (FSSAI, 2017). In the present study both cambodge and kokum butter recorded higher value for iron content when compared to milk butter.

5.3. Fatty acids profiling of butter

The GCMS analysis of three accessions of each cambodge and kokum butter revealed the presence of six major constituents. Among the different fatty acids, stearic acid and oleic acid were predominant followed by palmitic acid, myristic acid, lauric acid and capric acid. The proportion of long chain fatty acids (C_{14}-C_{18}) was higher than short chain fatty acids (C_4-C_{12}) in both cambodge and kokum butter. This was in opinion with the earlier studies of Ramachandran HD (2014), Niveditha (2013) and Parthasarathy *et al*. (2014).

Significant difference was observed in stearic acid content of cambodge (45.1 %) and kokum butter (58.08 %) extracted using petroleum benzene. The concentration of stearic acid was high in both cambodge and kokum, hence it may be in be used in manufacture of detergents, soaps, shaving creams, shampoo and other cosmetic products.

Stearic acid is an important saturated fatty acid found in cambodge and kokum butter. Stearic acid is stable at high temperature. Due to these properties, the butter of both species are solid at room temperature and used as supplements in food products such as margarine *etc*. Stearic acid has more role in preparation of margarine in such a way the cambodge and kokum seed butter finds properties in margarine preparation.

Significantly high composition of oleic acid was recorded in cambodge butter when compared to kokum butter extracted using both petroleum ether and petroleum benzene. The butter with high oleic acid composition could be useful in preservation of food by making it less susceptible to spoilage. Oleic acid may reduce the progression of adrenoleukodystrophy. It is a a fatal brain and adrenal gland disease as per Rizzo *et al*. (1986). The hypotensive properties of olive oil is due to presence of oleic acid in it (Teres *et al*., 2008). The presence of oleic acid in food could be useful in preventing heart related diseases as well as it also helps in reducing cholesterol. Due to presence of oleic acid in cambodge and kokum butter, it can be very well used in pharmaceutical sectors.

In the present study, palmitic acid was ranged from 0.760 to 3.056 per cent in cambodge accessions. Whereas in kokum, it was ranged from 1.814 to 4.544 per cent. According to Jagtap *et al*. (2015), palmitic acid concentration in kokum butter was 3 per cent. Palmitic acid is an ionic surfactant that gives the body a pleasant sensation. Hence, it is primarily used in the manufacture of cosmetics, soaps and releasing agents like paraffin wax. The most common saturated fatty acid found in plant and animal lipids is palmitic acid. Kokum butter has the ability to soften skin and heal ulcerations, fissures of lips, hands and soles of feet making it popular in skin care products. Palmitic acid aids in the control of obesity and the recovery of some reproductive abnormalities (Scott *et al*., 1988). In another study by Stephen *et al*. (2009) opinioned that diet rich with palmitic acid would be beneficial to cure diabetes.

Myristic acid, lauric acid and capric acid was recorded in smaller amount in both cambodge and kokum butter. The consumption of myristic acid had a positive impact on cardiovascular health by accumulating good fat in the body (Chowdhury *et al*., 2016). Lauric acid is believed to be used in treating viral infections like influenza, swine flu, avian flu, common cold *etc*. Capric acid is widely used in food as an artificial flavour, natural antimicrobial sanitizer in food processing plants and certain personal care products and as a mineral oil solubilizer it is been used.

5.4. Organoleptic evaluation of butter

Organoleptic qualities of extracted butter of both cambodge and kokum were evaluated in comparison with milk and cocoa butter. All these four types of butter were evaluated using nine point hedonic scale. A panel of judges evaluated seven quality attributes of butter like appearance, colour, flavour, texture, taste, after taste and overall acceptability.

Among different types of butter, milk butter (60.7) scored maximum followed by cocoa butter (53.48), kokum butter (51.96) and cambodge butter (51.36). Among the kokum and cambodge butter, kokum butter was rated high with mean score for quality attributes like appearance (7.69), flavour (7.16), texture (7.47) and after taste (7.55). Whereas cambodge butter secured high mean score for colour (7.82), taste (7.53) and overall acceptability (7.15).

5.5. Organoleptic evaluation of value added products

Organoleptic qualities of burfi and cake prepared using milk, kokum, cambodge and cocoa butter were evaluated following nine point hedonic scale.

Burfi, a type of basic fudge from Indian cuisine was made using four types of butter. Among the four butters used, burfi using milk, kokum butter, cambodge and cocoa butter obtained the total score of 57.34, 52.46, 55.48 and 48.47, respectively. Burfi prepared using milk butter was most preferred followed by kokum, cambodge and cocoa butter. For quality attributes like appearance and colour, the mean score was recorded highest in burfi prepared using cambodge butter when compared to milk, kokum and cocoa butter. According to Nair (2002), burfi prepared with cocoa mass after roasting of cocoa beans for 5 minutes was highly accepted than unroasted beans. Similarly in the present study, seeds of cambodge and kokum were roasted before extraction of butter.

Cake prepared using four types of butter was evaluated organoleptically for seven quality attributes. The highest total score was for cake prepared using milk butter (57.2), followed by cake prepared using kokum (54.53), cambodge (53.11) and cocoa butter (45.83 %). Among the quality attributes the mean score was recorded higher except texture for cake prepared using kokum butter when compared to cake prepared using cambodge butter. In the cake preparation, kokum and cambodge butter can be very well replaces with cocoa butter. During baking, it was observed that cake prepared using kokum butter had desirable texture. It also added to the softness of the cake as well as its rising properties. Fresh kokum cakes were most accepted by the panel of judges and the storability of cake prepared using kokum butter was less accepted.

Sweet dishes namely *holige* and *pancha kajaye* were prepared using cambodge butter in Uttara kannada and Dakshina kannada districts of Karnataka reported by Abraham *et al*. (2007). Reddy and Prabhakar (1994) reported both kokum fat and phulwara butter fractions were used in chocolate preparations as cocoa butter extenders. Phulwara butter fraction was mixed with Kokum fat in various amounts to produce wide range of hard butters with varying melting qualities. Blends with larger levels of Kokum fat were tougher in hotter climates, making them ideal for use in chocolate and confectionery. Maheshwari and Reddy (2004) reported that cocoa butter was replaced with kokum butter up to 15 % to increase the hardness of chocolate in hot regions.

SUMMARY

6. SUMMARY

The present work entitled "Physico-chemical and nutritional analysis of seed and seed-butter of *Garcinia* spp." was carried out to characterize the physico-chemical and nutritional properties of seed and seed-butter of cambodge and kokum. The experiment was conducted selecting three accessions of each cambodge and kokum. Among the three accessions of cambodge one each was selected from college farm, Department of Plantation, Spices, Medicinal and Aromatic crops; College Orchard, Department of Fruit Science, College of Agriculture, Vellanikkara as well as ICAR-NBPGR, Regional Station, Vellanikkara. In case kokum, one accession was collected from College Orchard, Department of Fruit Science, College of Agriculture, Vellanikkara and two accessions was selected from ICAR-NBPGR, Regional Station, Vellanikkara. Seeds from these accessions were collected during the peak period of fruiting. Seeds were separated from the fruits and utilized as research material. Analysis on biochemical and nutritional properties of seeds and extraction of butter using different solvents was done. Physico-chemical and nutritional analysis of butter and their fatty acid profiling was carried out. Butter of both cambodge and kokum butter were subjected to organoleptic evaluation in comparison with milk and cocoa butter. The products *viz*. burfi and cake were prepared using both *Garcinia* species butter and this was compared with products of milk and cocoa butter. The salient features of the present study are summarized here under.

6.1. Biochemical and nutritional analysis of seeds

There was significant difference was recorded among the accessions of cambodge seed powder for all the biochemical characters. In case of kokum, all accessions exhibited significant difference for biochemical characters except total fat. Moisture content of cambodge seed powder (5.76 %) was significantly lower than that of kokum seed powder (11.45 %). No significant difference was observed between cambodge and kokum seed powder for starch content. Total carbohydrates content was significantly higher in cambodge (13.33 g per 100 g) when compared to kokum seed powder (4.56 g per 100 g). Protein content was significantly higher in cambodge (1.28 g per 100 g) than that of kokum seed powder (0.42 g per 100 g). Significant difference was recorded for fat content between the *Garcinia* species studied. Kokum seed powder (40.28 %) was recorded significantly higher fat content when compared to cambodge seed butter (38.07 %). Significantly higher percentage of total ash or total minerals was recorded in cambodge seed powder (2.53 %) than that of kokum seed powder (2.36 %). Kokum seed powder (4.55 %) had significantly higher dietary fibre to that of cambodge seed powder (1.57 %).

In case of cambodge and kokum, all accessions exhibited significant difference for nutritional characters except iron. Calcium content in seed powder was significantly higher in cambodge (163.20 mg per 100 g) when compared to kokum (48.30 mg per 100 g). Significantly higher iron content was observed in cambodge seed powder (16.20 mg per 100 g) when compared to kokum seed powder (14.30 mg per 100 g). Potassium content of kokum seed powder (608.50 mg per 100 g) was recorded significantly higher to that of cambodge seed powder (478.40 mg per 100 g). Sodium level of kokum seed powder (32.10 mg per 100 g) was significantly higher when compared to cambodge seed powder (15.00 mg per 100 g)

6.2. Extraction of butter from *Garcinia* species using different solvents

The extraction of butter from seeds of cambodge and kokum were recorded higher in solvent extraction method when compared to hot water skimming method. The quantity of butter significantly varied using different solvents *viz.* acetone, petroleum ether and petroleum benzene. In cambodge, significantly high butter yield was recorded using acetone (44.98 %) which was followed by petroleum ether (38.40 %) and petroleum benzene (37.25 %). Whereas in kokum, acetone (49.61 %) recorded significantly higher butter yield when compared to petroleum benzene (42.25 %) and petroleum ether (40.28 %). The appearance of butter was good extracted using petroleum ether. Petroleum ether is most accepted food grade solvent for plant based extraction.

6.3. Physico-chemical and nutritional analysis of butter

In cambodge, all the accessions exhibited significant difference for physico-chemical properties except refractive index, peroxide value and ester value. Whereas in kokum, all the accessions showed significant difference for physico-chemical properties expect ash content, iodine value and peroxide value. Both cambodge and kokum butter were solid at room temperature. The colour of *Garcinia* species butter (cambodge & kokum) was yellowish white. Moisture content of kokum butter (0.15 %) was recorded significantly lower when compared to cambodge butter (0.40 %). Oil or total fat content of butter was significantly higher in kokum (99.85 %) than that of cambodge (99.60 %). There was no significant difference observed for refractive index, pH and acid value between cambodge and kokum butter. The melting point of cambodge butter (39.17 °C) was significantly when compared to kokum butter (37.94 °C). The boiling point of both cambodge and kokum butter were more than 300 °C. Both cambodge and kokum butter were insoluble in water and alcohol. Butter of both species was soluble in chloroform. Saponification was significantly higher in cambodge (188.70 mg KOH per g) than that of kokum butter (180.00 mg KOH per g). Significantly difference was observed for iodine value between *Garcinia* species butter. Cambodge butter (57.05 g per 100 g) recorded significantly higher when compared to kokum butter (37.95 g per 100 g). Peroxide value of cambodge butter (4.39 meq per kg) was significantly lower to that of kokum butter (5.81 meq per kg). Ester value was significantly higher in cambodge (183.20 mg KOH per g) than that of kokum butter (174.40 mg KOH per g).

In cambodge, all accessions observed significant difference for nutritional composition. Whereas in kokum, all accessions exhibited significant difference for nutritional composition except sodium. There was no significant difference was observed for calcium and iron content between cambodge and kokum seed butter. Potassium content of cambodge seed butter (32.80 mg per 100 g) was significantly higher to that of kokum seed butter (18.30 mg per 100 g). Sodium content of kokum butter (17.60 mg per 100 g) was significantly higher when compared to cambodge butter (14.90 mg per 100 g).

6.4. Fatty acid profiling of cambodge and kokum seed butter

GCMS analysis has been found six major fatty acids *viz*. stearic acid, oleic acid, palmitic acid, myristic acid, lauric acid and capric acid in both cambodge and kokum butter. Stearic acid and oleic acid were predominant in cambodge and kokum butter. Stearic acid was recorded higher in kokum butter to that of cambodge butter extracted using acetone, petroleum ether and petroleum benzene. Whereas oleic acid was recorded higher in cambodge butter when compared to kokum butter. Stearic acid concentration in cambodge and kokum ranged from 36.853 to 50.761 per cent and 32.056 to 62.524 per cent, respectively using different solvents. The concentration of oleic acid varied from 46.276 to 61.372 per cent and 35.593 to 65.427 per cent in cambodge and kokum, respectively. Palmitic acid concentration in cambodge and kokum butter ranged from 0.760 to 3.056 per cent and 1.814 to 4.544 per cent, respectively. The compounds such as myristic acid, lauric acid and capric acid were also identified in smaller proportion in cambodge and kokum butter.

6.5. Organoleptic evaluation of butter and their products

Total organoleptic score of milk butter (60.7) was observed superior. Whereas total organoleptic score of cocoa butter (53.48) was higher, which was closely followed by kokum (51.96) and cambodge butter (51.36). The mean rank score of milk butter was significantly superior compared to the other three butters. Mean rank score for quality attributes like appearance and colour were significantly superior in cambodge and kokum butter when compared to cocoa butter.

Total organoleptic score of milk burfi (57.34) was found superior, which was closely followed by cambodge (55.48) and kokum burfi (52.46). A low total score was recorded for cocoa burfi (48.47). The mean rank score of cambodge burfi (3.03) for quality attribute like colour was significantly superior when compared to burfi prepared using other butters. Mean rank score of burfi prepared with cambodge and kokum butter was significantly higher for quality attributes like appearance, flavour, texture, taste, after taste and overall acceptability when compared to cocoa burfi.

Total organoleptic score of milk butter cake (57.2) was observed superior which was closely followed by kokum (54.53) and cambodge cake (53.11). A low total score was attained for cocoa butter cake (45.83). Mean rank score of all quality attributes *viz.* appearance, colour, flavour, texture, taste, after taste and overall acceptability were significantly higher in both kokum and cambodge butter cake when compared to cocoa butter cake.

From the study, it can be concluded that effective use of cambodge and kokum seeds for extraction of butter or oil on a commercial scale would encourage small scale industries in setting up of extraction units and in the value addition sectors. Cambodge and kokum seeds yields butter of food grade. It can be comparable with milk and cocoa butter in all biochemical and nutritional characters. Plenty seeds of both *Garcinia* species after fruit rind collection go as waste and only a limited amount of seeds utilized for seedling production. Making use of seeds of both species to extract seed butter helps in conversion of waste produce into value added form. The defatted seed powder has place in preparation of cattle feed or it can be converted into enriched compounds. Cambodge and kokum butter are rich in fatty acids *viz.* stearic acid, oleic acid and palmitic acid. Due to these properties, it can be recommended to use in pharmaceutical and cosmetic sectors. Value added products like burfi and cake prepared using cambodge and kokum butter exhibited close quality attributes with milk and cocoa butter products. Hence cambodge and kokum butter can be blended or substituted with milk or cocoa butter in the food preparations.

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PHYSICO-CHEMICAL AND NUTRITIONAL ANALYSIS OF SEED AND SEED-BUTTER OF *GARCINIA* SPP.

By

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ABSTRACT OF THE THESIS

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ABSTRACT

The genus *Garcinia* belonging to the family Clusiaceae is an underutilized perennial tree found throughout the tropics of Asia and Africa. *Garcinia* species such as cambodge (*Garcinia gummi-gutta*) and kokum (*Garcinia indica*) are commercially exploited as condiments to flavour a range of food preparations. Cambodge is grown widely in the homestead of Kerala, whereas kokum is cultivated as a traditional homestead crop in the Konkan region of Maharashtra, Goa and coastal and southern interior parts of Karnataka. The economic part of both cambodge and kokum is dried fruit rind. Plenty of seeds after fruit rind collection go as waste and only limited seeds are utilized for seedling production. It is essential to evaluate the seeds for biochemical and nutritional factors, butter recovery as well as physico-chemical and nutritional properties of butter. In this context, the present study was undertaken with the objective to characterize the physico-chemical and nutritional properties of seed and seed butter in cambodge and kokum. The experiment was carried out in the Department of Plantation, Spices, Medicinal and Aromatic Crops, College of Agriculture, Vellanikkara. Three high yielding accessions of each cambodge and kokum were selected from the department farm and college orchard of the College of Agriculture, Vellanikkara, and ICAR-NBPGR, Regional Station, Vellanikkara.

The significant difference was recorded among the accessions of cambodge and kokum seed powders for most of the biochemical characters and also between cambodge and kokum. The total carbohydrates, protein and total ash contents were significantly higher in cambodge seed powder (13.33 g/100 g ,1.28 g/100 g and 2.53 %) when compared to kokum seed powder (4.56 g/100 g, 0.42 g/100 g and 2.36 %), respectively. Whereas, in kokum seed powder significantly higher moisture content (11.45 %), total fat (40.28 %) and dietary fibre (4.55 %) were recorded.

In the case of cambodge and kokum seed powders, all accessions exhibited significant differences in nutritional characters except for iron content. There was a significant difference in mineral composition between the cambodge and kokum seed powders. Calcium and iron contents were significantly higher in cambodge seed powder (163.20 and 16.20 mg/100 g) than that of kokum seed powder (48.30 and 14.30 mg/100 g), respectively. Whereas, potassium and sodium contents were

significantly higher in kokum seed powder (608.50 and 32.10 mg/ 100 g) when compared to cambodge seed powder (478.40 and 15.00 mg/ 100 g), respectively.

The recovery of butter ranged from 37.25 (petroleum benzene) to 44.98 (acetone) per cent in cambodge and 40.29 (petroleum ether) to 49.61 (acetone) per cent in kokum using different solvents. Butter recovery in cambodge and kokum were found higher in acetone compared to other solvents. But the appearance of butter was found good when extracted using petroleum ether. In both cambodge and kokum, butter extracted through the hot water skimming method ranged from 22 to 25 per cent.

The seed butter extracted using petroleum ether was employed for physico-chemical analysis. Among the cambodge accessions, a significant difference was recorded for the physico-chemical properties of butter except for refractive index, peroxide value and ester value. Whereas in kokum, all the accessions showed significant differences in physico-chemical properties except for ash content, iodine value and peroxide value. Moisture content, melting point, ash content, saponification value, iodine value and ester value were significantly superior in cambodge butter (0.40 %, 39.17 °C, 0.139 %, 188.70 mg KOH/g, 57.05 g/100 g, 183.20 mg KOH/g) compared to kokum butter (0.15 %, 37.94 °C, 0.116 %, 180.00 mg KOH/g, 37.95 g/100 g, 174.40 mg KOH/g), respectively. The oil content and peroxide value of butter were significantly higher in kokum (99.85 % and 5.81 meq/kg) than that of cambodge (99.60 % and 4.39 meq/kg), respectively. No significant difference was observed for refractive index, pH and acid value between cambodge and kokum butters.

Among the cambodge accessions, significant difference was observed for nutritional composition in the seed butter. In kokum accessions, significant difference was recorded for nutritional composition except for sodium content. The significantly higher value for potassium content was recorded in cambodge (32.80 mg/100 g) compared to that of kokum (18.30 mg/100 g). Whereas for sodium content, significantly higher value was recorded in kokum butter (17.60 mg/100 g) when compared to cambodge butter (14.90 mg/100 g). No significant difference was recorded in calcium and iron contents between cambodge and kokum butters.

The fatty acid profiling of seed butter in cambodge and kokum exhibited six prime fatty acids. In cambodge and kokum butters, high percentage (99) of long-chain fatty acids *viz.* stearic acid, oleic acid and palmitic acid were recorded. Stearic acid in cambodge butter ranged from 36.06 (acetone) to 50.76 per cent (petroleum ether), whereas in kokum butter, it ranged from 32.06 (acetone) to 62.54 per cent (petroleum benzene) using different solvents. Oleic acid percentage in cambodge butter ranged from 46.28 (petroleum ether) to 61.37 (acetone) per cent, and in the case of kokum butter it ranged from 35.59 (petroleum ether) to 65.43 per cent (acetone). The compounds such as palmitic acid, myristic acid, lauric acid and capric acid were also identified in smaller proportions in cambodge and kokum butter.

In the organoleptic evaluation, cambodge and kokum butters were compared with milk and cocoa butters, which revealed that milk butter was superior in all the organoleptic qualities. Whereas, cambodge and kokum butter were significantly superior in quality attributes like appearance (7.69 and 7.53) and colour (7.58 and 7.82) compared to cocoa butter (6.82 and 6.64), respectively. Value added products *viz.* burfi and cake were prepared using cambodge, kokum, cocoa and milk butters. Organoleptic evaluation of value added products revealed that the burfi and cake prepared using milk butter was most accepted with a total score of 57.34, which was followed by burfi and cake of cambodge (55.48 and 53.11) and kokum (52.46 and 54.53), respectively. Both cambodge and kokum seed yield butter of food grade having high biochemical and nutritional properties which can be used in the food, pharmaceutical and cosmetic sectors.