PREPARATION, QUALITY EVALUATION AND SHELF LIFE STUDY OF *DHULE ACHAR*, A TRADITIONAL PICKLE OF NEPAL

by

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Approval Letter

This *dissertation* entitled *Preparation, Quality Evaluation and Shelf Life Study of Dhule Achar, A Traditional Pickle of Nepal* presented by *Basanta Raj Adhikari* has been accepted as the partial fulfillment of the requirements for the B. Tech. in Food Technology.

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Abstract

Preparation, quality evaluation and shelf life study of *dhule achar,* a traditional pickle of Nepal was initiated by conducting a survey based on traditional method. A survey was conducted about the preparation method of *dhule achar* among the different communities of people. Information on recipe, preparation method, uses, benefits and origination of *dhule achar* were collected. From survey information, variation was found regarding the raw materials (sesame, niger, flax, perilla and pumpkin seeds), recipe and methods of preparation. So, a study was carried out on the effect of variation of five oilseeds on the sensory quality of the product. From the sensory evaluation on different attributes (color, taste, flavor, texture and overall acceptance), *dhule achar* prepared by using equal proportion of all oilseeds, was found to be best as compared to the product made from individuals oilseeds. Then again the products were made by varying the proportions of the each oilseed. The best formulation selected from the sensory analysis was the product containing higher proportion of pumpkin seeds (30%) and it was analyzed for its proximate composition and some minerals (Ca, Fe, K and Na). The proximate analysis showed moisture (12.26±0.28%), crude protein (24.3±0.19%), crude fat (30.80±0.24%), ash (7.5±0.13%), crude fiber (4.67±0.13%) and total carbohydrate (20.47±0.98%). Similarly, mineral contents were found to be calcium (242.56±6.02mg), iron (15.32±0.76 mg) sodium (2174±7.81 mg) and potassium (673±2.34 mg). The shelf life of the product can be extended for few months by keeping the product in PP pack (80μm) at ambient condition (25±3˚C).

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Part I

Introduction

1.1 General introduction

Food is essential for human beings. People have different taste of foods and different food habits depending upon their religion, ethnicity, geography, climatic, seasonal, educational, economical, rural and urban situations (K.C., 2007). Specific eating habits play an important role in the traditional habits of many cultures. The use of particular food ingredients and food preparation methods has been passed on from one generation to the next, and are nowadays referred to as 'traditional foods' (Trichopoulou *et al*., 2006).

In order that indigenous food can be promoted in a more sustainable way, it is imperative that the knowledge of traditional food is encouraged. Knowledge of traditional food is important for sustaining their development and utilization. It is important for people to know the prevailing traditional food in their areas and how they can be improved for better sustainable food security. Research has proved that indigenous food plays an important role in raising the economy of rural population (Ohiokpehai and Ramosweu, 1999).

Nepal is a country of ethnical, cultural, religious and linguistic diversity. So, also diverse kinds of traditional foods can be found in Nepal, which the people have inherited from their ancestors. Many traditional foods have no recorded manual and the process and skill of preparation is verbally handed over the generation (Kharel *et al*., 2010).

Dhule achar is the traditional pickle indigenous to Nepal and prepared by using different oilseeds (perilla, niger, sesame, flax and pumpkin seeds, etc) either individually or by mixture of these oilseeds using spicy ingredients like turmeric, ginger, coriander, cumin, chilli, salt and acid *(chuk amilo*). These all are blended after being roasted and powdered. It is preferred in different communities of Nepal and most popular in *Chhetri* and *Bahun* community. It is specially popular in hilly and mountainous region. Its origination is not fixed and supposed to be started from time immemorial. In hilly region, *dhule achar* is mostly prepared from pumpkins seeds and niger seeds as major raw material. *Aathpahariya Rai* communities prefer the mixture of pumpkin seeds and niger seeds. *Sherpa* tribes prepare *dhule achar* from mixture of pumpkin seeds, niger seeds and perilla seeds. In Terai region, *dhule achar* is mainly prepared from flaxseeds and sesame

seeds. Pumpkin seeds are also used if available. It is usually served as the chutney along with meal. It acts as good appetizer and it has high nutritional and medicinal value (Personal communication, 2012).

1.2 Problem statement

Nepal is rich in its traditional foods. Some of them are nutritious, some are used as appetizer, and some have medicinal value. But nutritionally rich traditional foods of Nepal are in verge of extinction due to the lack of documentation and commercially promoted products are dominating the market of *dhule achar*. Morover, many traditional foods are facing problems due to introduction of western foods and consequent acculturation. So it is one of the extincting traditional foods with immense nutritional and medicinal value. Despite, the immense nutritional, functional and medicinal value, their manufacturing process, functional properties, nutritional quality, shelf life have not been studied yet and no initiative has been undertaken for their documentation, standardization and commercialization. Spices are used as flavorings and condiments only while it can be a good source of minerals, antioxidant and some medicinal functions (Wildman, 2002).

Research, study and documentation of *dhule achar* is however rare. Its formulation, optimization in processing and preparation method is still under scientific level. The preparation and consumption of *dhule achar* is confined to household level and not still launched in market. In household level, it is not packed in good condition therefore liable to deterioration. The selection of raw material and ingredients varies from place to place and household to household.

1.3 Justification of the work

Digestion of food is major prior when the term consumption ahead. Some foods besides providing nutritional, functional or medicinal value also play role to improve consumption and even digestion. *Dhule achar* is a good appetizer and no doubt enhances the consumption rate of other foods. As the ingredients used in *dhule achar* possess antioxidants, the product may be good medicinal food adjunct.

Spices have various effects when used in foods: not only do they impart flavor, pungency, and color characteristics, they also have antioxidant, antimicrobial, pharmaceutical, and nutritional properties. Several studies have been conducted on the nutritive biochemical effects of the intake of spice components. The majority of herbs and

spices constitute important bioactive secondary metabolites which possess versatile pharmacological and medicinal properties (Hirasa and Takemassa, 1998). Tradition attaches all manner of benefit to every spice, condiment and herb, and they are important ingredients in pharmacopoeias of the Indian system of medicine including Ayurvedic, Siddha and Unani systems (Kochhar, 1999).

Dhule achar is one of the undocumented traditional foods with immense nutritional and medicinal value. Preparation method of *dhule achar* is limited to the certain community only and there lies some variation regarding the ingredients used but no work has been carried out on its preparation method, recipe formulation and standardization. Furthermore, *dhule achar* is prepared from locally available ingredients like perilla, niger, sesame, flax, pumpkin seeds, ginger, coriander, cumin, *chuk amilo* etc. using the simple household technology. It can be a rich source of antioxidants, polyphenols and micronutrients. So, promoting the use of *dhule achar* in daily diet in community level may be helpful in solving the overall micronutrients deficiency diseases of Nepal. Thus, study on nutritional quality of such indigenous food is of tremendous importance to document, standardize, formulate and commercialize the cheap traditional product. Hence, formulation, preparation, quality evaluation and shelf life study of *dhule achar* was undertaken.

1.4 Objective

1.4.1 General objective

The general objective was to formulate and prepare the traditional pickle *dhule achar* based on survey and evaluate its nutritional quality and storage stability.

1.4.2 Specific objectives

1. To survey on traditional methods of *dhule achar* preparation.
2. To prepare *dhule achar* from different oilseeds varieties and to develop superior formulations by varying their proportion.
3. To select the best formulation based on sensory analysis.
4. To determine the proximate constituents and some mineral contents in final product.
5. To evaluate the storage stability of *dhule achar.*

1.5 Significance of the study

The findings of this work can be further extended to document the traditional nutritious food of Nepal. If the study on nutritional quality and trial feeding shows encouraging results, *dhule achar* can be recommended as nutritious appetizer in hotels, resturants, communities and household levels to promote this pickle. This work would provide the basis for the further work in this the industrial and commercial field.

1.6 Limitations

1. Amino acid profile of the prepared product couldn't be analyzed.
2. Sufficient literature about *dhule achar* couldn't be collected.
3. Control sample couldn't be arranged.
4. Antioxidant activity of the product couldn't be analysed due to lack of time.

Part II

Literature review

2.1 Traditional foods of Nepal

"Food" means any unprocessed, semi-processed, processed or produced food or drinking substance which the human being generally consumes and drinks, and includes any species, food additives, color or flavor to be used in any food or drinking substance (Food Act, 2023).

Traditional foods are those foods originating locally in an area with respect to the country, region, district or sub-district and concern with religions, castes, ethnics etc. The definition includes all indigenous food products (IPGRI, 1992).

Traditional foods have played a major role in traditions of different cultures and regions for thousands of years. They include foods that have been consumed locally and regionally for an extended time period. Preparation methods of traditional foods are part of the folklore of a country or a region. However, some traditional foods are at risk of disappearing due to altered lifestyles. Therefore, it is important to study and document traditional foods to sustain their important elements (Trichopoulou *et al*., 2006).

Nepal is a country of different cultures, religions and races. They have their own food habits oriented from their ancestors since time immemorial. They have their own traditional methods of food preparation and preservation transferring from generation to generation. Traditional foods do not have any written documents about their preparation, processing and quality control methods. The food preparation method is learnt by "doing and learning" system, which is taught by mother to the daughter. The quality checking is performed by using their senses e.g., by looking, smelling, touching and chewing (for taste). There is no standard scientific measuring system in such foods. In Nepal, the usual indigenous mode of pre-processing practices generally consists of cooking, frying, boiling, roasting, grinding etc. (K. C., 2007).

We have wide variety of traditional foods. Some of them are highly nutritious like *sattu, kinema*, *yangbean* etc. Also, we have some example of indigenous practices of medicated preparation of foods such as *chyawanprash, Jatikari, battisa, sattu, asthamandal, dhule achar* etc. some traditional food are taken as snacks, some as appetizer and some have religious value (Regmi, 1980).

Among the traditional products, few are highly commercialized and promoted, some of them are semi commercialized and many of them are not yet documented and are limited to the specific community. *Churpi, gundpak, pustakari, chiura, bhuja* are some of the traditional products which are successfully commercialized. These days *selroti, bhakka, gundruk, jand, tongba* are sold at local markets. *C*hyo*la, sekuwa, kachilla, dindo* are some of the traditional food promoted to the restaurant level (Kharel *et al*., 2010).

2.2 *Dhule Achar*

2.2.1 Introduction

In Nepal, a variety of chutneys and pickles in large volumes based on vegetables, pulses and spices are consumed alongwith rice and breakfast items. Literatures are available on development, optimization and standardization of several pickles and chutneys based on the various raw materials available during different seasons. Interestingly, there was no literature available on utilization of oilseeds in such food adjuncts. *Dhule achar* is a traditional pickle prepared from different oilseeds available in Nepal. It is one of the most appreciated pickle preferred by almost all communities in Nepal. It is prepared by mixing individually or in mixed proportion of roasted and powered pumpkin seeds, sesame seeds, flax seeds, niger seeds, perilla seeds etc. with salt, chilli, acid and other selected spice ingredients. The process of manufacture and ingredients used depends on the availability of raw material and differ from place to place and household to household. It is consumed along with rice breakfast items like bread etc. since the product is an intermediate moisture food, it has a shelf life of several month if properly stored.

2.2.2 Origin and history

Dhule achar is a ancient pickle of Nepal. There is no such authentic document regarding the history of *dhule achar*. However, it has been used in festive occasion such as *puja, kajkriya, bratabandha, bibah*, etc. since time immemorial. Being dry product it is easy to carry during long journey.

2.2.3 Uses and benefits

Dhule achar is generally served along with rice. It can be consumed by all age group of people. It is good appetizer and aids in digestion process. This food is supposed to have high nutritional and medicinal value. It also helps to cure constipation and improves digestive system. *Dhule achar* has a characteristic mouth feel with good flavor and taste

when consumed with cooked rice. It is digestible, proteinous and energy rich food. Its high medicinal value is supposed to enhance immunity power of a person. If it is consumed daily in small amount, it alleviates arthritis and diabetes (Personal communication, 2012).

2.3 Technology of preparation of *dhule achar*

Dhule achar is the traditional, nutritious, powdery pickle. It is a prepared by using different oilseeds such as pumpkin seeds, perilla seeds, niger seeds, sesame seeds and flaxseeds mixed with, acid (*chuk amilo*) and spices (ginger, turmeric, chilli, cumin, coriander and salt). Traditionally, spices are cleaned, sun dried and then grinded in *okhali*. Similarly, these oilseeds are also separately cleaned, roasted under flame then ground using *okhali*. Each ingredient are processed separately and later on blended in the different proportion based on available of raw materials (Personal communication, 2012). The general flowchart for the traditional method of *dhule achar* preparation is shown in Fig. 1.1.

2.3.1 Cleaning

Ingredients are cleaned to remove foreign materials and sort out the damaged particles. Foreign material common to oilseeds and spices are mud, clay, stone, foreign, rodents excreta etc. Similarly, spices are likely to contain mud, stone, sand etc. Further cleaning makes food safe to eat and also improves aesthetic value of finished products. Similar method of cleaning can be used for oilseeds and spices being all low moisture and abrasion resistant food. General methods of cleaning for oilseeds and spices include screening, abrasions, aspirations, magnetic method and electrostatic method (Kent, 1983).

2.3.2 Drying

Spices are sun dried to produce a friable, readily milled stable product. Further drying reduces the moisture content and protect from the mold infestation and risk of aflatoxin production. In some spices, flavor is intensified through drying because of the elimination of most moisture. This leaves a greater concentration of the low volatile compounds that give stronger flavor but fewer aromas due to the loss of the volatile constituents. Dried spices can better withstand higher temperatures and processing conditions than fresh spices (Raghavan, 2006).

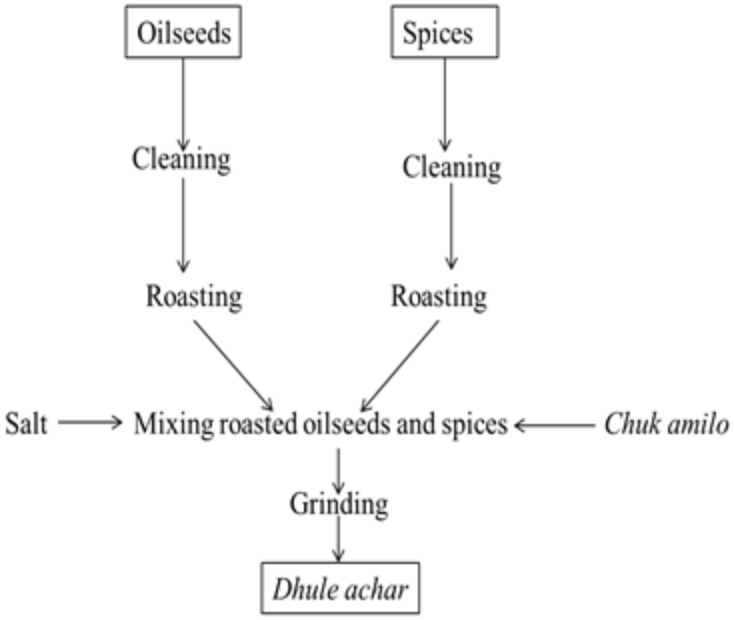

Fig. 1.1 Traditional method of *dhule achar* preparation

Multistage drying is generally preferred for spices to better retain its flavors. Smoke drying is also practiced for drying some spices like ginger traditionally. Best drying temperature for ginger is 75^0C for 3 hrs then drying at 65^0C till moisture content decreases to 10% (Govindarajan, 1980).

2.3.3 Roasting

Roasting reduces the moisture content, thereby concentrating the food value. Roasting also enhances acceptability by imparting a nutty flavor and brown color to the food (Krantz *et al.*, 1983). It also helps in grinding and sieving process. The optimum temperature and time roasting from sesame to obtain the most antioxidants and total phenolic content was 200º C for 20 min (Janat *et al.*, 2010). During roasting, volatile concentrations increase significantly which has been attributed to the maillard reaction, lipid oxidation and pyrolysis. Many studies have been conducted on volatile flavor formation during roasting reactions in model food and various food matrices including coffee (Hashim and Elkiey, 1962), cocoa (Huang *et al.*, 2004), peanuts, almonds and pumpkin seeds (Siegmond and Murkovic, 2004). Aldehydes from lipid oxidation that contribute to the aroma of roasted pumpkin seeds included pentenal, hexanal and 2-heptenal, and increase during roasting conditions (Siegmond and Murkovic, 2004).

2.3.4 Grinding and sieving

By reducing the food into a powder form results in a more homogenous product. Sieving the powder gives equal size particle to give better textural property (Krantz *et al.,* 1983). Ground spices have better dispersibility in food product than whole spices. Grinding partially breaks down the cellular matrix of the spice and releases some volatile oils. Spices are ground using low temperature to preserve its volatile constituents (Raghavan, 2006). Pamidighantam *et al*., (2011), reported that roasted flaxseeds powder sieved through 500µ gave palatable chutney product.

2.3.5 Mixing

Mixing is done to provide homogeneity to the product. Mixing of dry solid food involves mainly three basic mechanisms. These are convection, diffusion and shear. Ribbon and screw mixers are also used for blending and mixing of dry product (Sahay and Singh, 1987).

2.3.6 Packaging

Dhule achar is lipid rich powdery product which is also rich in flavor. So it requires special packaging to extend its storage life. Following moisture pick-up certain physical, chemical and biological changes will take place and quality degradation will begin. Air may reduce the flavor quality of the product. Various packaging materials may be used but they should be moisture and air tight, e.g., glass containers, aluminum-foil, sealed tin containers and plastic bags. Packaging methods generally used for dried powder are vacuum packaging and inert gas packaging but these methods are costly (Krantz *et al.,* 1983).

2.4 Ingredients and their nutritive value

2.4.1 Sesame seeds

Sesame seed (*Sesamum Indicum*) is one of the world's most important and oldest oilseed crops known to man (Garbia-Abau *et al*., 2000). Sesame is grown mainly in the developing tropical and subtropical areas of Asia, Africa, South and Central America (Brito and Nunez, 1982). Sesame is called the "Queen of the oilseeds crops" because of its high oil yield, mildness and pleasant taste (Johnson *et al*., 1979). Sesame use has long been

regarded in the oriental dishes as a healthy food which increases energy and prevents aging (Namiki, 1995). The maintenance of dietary minerals homeostasis in the human body is very important, since they are part of enzymes involvement in fundamental biological processes. Sesame seed is not only a good source for edible oil but also is widely used in baked goods and confectionery products (Namiki, 1995). Budowski (1964) noted that sesame oil is much more stable against oxidative changes than other vegetable oils. Its remarkable stability is due to the presence of sesamin, sesamolin, sesaminol, sesamol, and α-tocopherol. Sesamin showed antioxidant (Yamashita *et al*., 2000), anticarcinogenic (Hirose, 1992), blood pressure lowering (Matsumura *et al.*, 1998), and serum lipid-lowering effects (Hirose *et al.*, 1991). Sesame has long been regarded in the orient as a health food for energy increasing and aging prevention (Hajimahmoodi *et al*., 2008).

The oil of the seeds of *Sesamum indicum* contains saturated and unsaturated acids (10-14% and 80-86%) respectively. The major saturated fatty acids in seed oil are palmitic (8-11%), stearic (4-6%) acids with small arachidic acid (max 1%). The main unsaturated fatty acids are linoleic (40-48%) and oleic (35-40%) acids (Teco, 2005). Its oil can be classified in theoleic-linoleic acid group. Linoleic acid which is one of the most important polyunsaturated fatty acids in human food because of its prevention of distinct heart vascular diseases (Boelhouwer, 1983). The detail chemical composition of sesame seeds is given in Table 2.1.

2.4.2 Pumpkin seeds

Pumpkin seeds, also known as pepitas, are flat, dark green seeds of the fruit pumpkin of the genus Cucurbita and the family Cucurbitaceae. Some are encased in a yellow-white husk, although some varieties of pumpkins produce seeds without shells (Ensminger *et al*., 1983). It has valuable dietetic and medicinal advantages besides being a source of edible oils, proteins and minerals of good quality (Yoshida *et al*., 2005). The content of vitamin E in pumpkin seed is very high and the main isomers are α- and γ-tocopherols (Idouraine *et al*., 1996; Murkovic and Pfannhauser, 2000). The seed itself can be eaten as a snack or ground as an ingredient of sauces. Pumpkin seed has also been used in traditional medicine with combination from several plants and herbs which contain fatty acids and phytosterols and are used in the treatment of benign prostatic hyperplasia. Pumpkin seed is also rich in plant sterols which have recently become of great interest due to the serum cholesterol-lowering effect .The seeds may also be beneficial against colon cancer (Zhang *et al*., 1994).

According to Alfawaz (2004), pumpkin seed oil consisted saturated fatty acid 27.73% and comprises of 16.41% palmitic acid and 11.14% stearic acid. The unsaturated fatty acid value was 73.03% and consisting mainly of 18.14% oleic acid and 52.69% linoleic acid. The highly unsaturated fatty acid composition of pumpkin seed oil makes it well-suited for improving nutritional benefits from foods. Pumpkin seed oil has been implicated in providing many health benefits. The most critical health benefit attributed to pumpkin seed oil is preventing the growth reducing the size of the prostate (Tsai *et al*., 2006). There is also evidence that suggests pumpkin seed oil can retard the progression of hypertension (Zuhair *et al*., 2000) and mitigate hypercholesterolemia and arthritis (Fahim *et al.*, 1995). Reduced bladder and urethral pressure and improved bladder compliance have been linked to pumpkin seed lipid components (Schilcher *et al.*, 1996). Pumpkin seed oil has been found to alleviate diabetes by promoting hypoglycemic activity (Fu *et al.*, 2006). Pumpkin seed oil has been found to provide a significant source of vitamin E (tocopherol) in Japanese diets (Imaeda *et al.*, 1999). Diets high in pumpkin seeds have also been associated with lower levels of gastric, breast, lung, and colorectal cancer (Huang *et al.*, 2004).

Dimethylsulfide, methanethiol and methional are considered to have the highest flavor impact of the sulfur compounds (Harper *et al.*, 2010) and have been identified in roasted pumpkin seeds (Siegmund and Murkovic, 2004). Pyrazines have been identified in roasted pumpkin seed which considered to be an important flavor component. At low levels, pyrazines have been associated with a sweet, nutty and typical roasted aroma. Siegmund and Murkovic (2004), identified five pyrazines, 2-methylpyrazine, dimethylpyrazine, 2-ethylpyrazine, 2-ethyl-5(6)-methylpyrazine and 2-ethyl-3, 6-dimethylpyrazine, in the headspace of roasted pumpkin seeds, and theorized to significantly contribute to the overall aroma of roasted pumpkin seeds. The detail composition of pumpkin seeds is given in Table 2.1.

2.4.3 Perilla seeds

Perilla seed (*Perilla frutescens*) belongs to the family Lamiaceae. These are oval or globe-shaped, and just over a millimeter in size. Externally, they are grayish-brown, with purple striations. The outer covering of a perilla seed is thin and fragile, and breaks easily. The seeds themselves are yellowish-white and have a slightly pungent taste. They are usually harvested in autumn when the fruit ripens, then dried in the sun. They can be used either

raw or after being parched, and are crushed or finely ground into powder (Ihara *et al*., 1998).

Total lipid contents of the five perilla seed ranges from 38.6 to 47.8% on a dry weight basis. The lipids consists of 91.2-93.9% neutral lipids, 3.9-5.8% glycolipids and 2.0-3.0% phospholipids. Neutral lipids consists mostly of triacylglycerols (88.1-91.0%) and small amounts of sterol esters, hydrocarbons, free fatty acids, free sterols and partial glycerides. Among the glycolipids, esterified sterylglycoside (48.9-53.2%) and sterylglycoside (22.1-25.4%) are the most abundant, while monogalactosyldiacylglycerol and digalactosyldiacylglycerol are present as minor components. Of the phospholipids, phosphatidylethanolamine (50.4-57.1%) and phosphatidylcholines (17.6-20.6%) are the major components, and phosphatidic acid, lysophosphatidylcholine, phosphatidylserine and phosphatidylinositol are present in small quantities. The major fatty acids of the perilla oil are linolenic (61.1- 64.0%), linoleic (14.3-17.0%) and oleic acids (13.2-14.9%) (Hyo-sun and Sung-Whan, 1994).

Some of the physicochemical characteristics and the tocopherol composition of perilla oil were determined. The seeds of the perilla plant are a good source of oil, at approximately 40% oil content. The seed consistently contains the highest proportion of omega-3 fatty acids, at 54-64%. The omega-6 component is usually around 14%, giving an omega-3 to omega-6 ratio of 3.9-4.6:1 (Longvah *et al*., 2000). Oleic acid (omega-9 component) is also present in perilla oil (Klein *et al*., 1999). The detail chemical composition of perilla seed is given in Table 2.1.

2.4.4 Flaxseeds

Flax seed (*Linum usitatissimum*) belongs to the family of Linaceae which is a versatile, blue-flowered crop. The seed is flat and oval with a pointed tip. The seeds have a crisp and chewy texture and a pleasant, nutty taste. It is a little larger than a sesame seed and measures about 4-6 mm and has recently gained good attention as a functional food due to the presence of omega 3- fatty acid and fibre. Flaxseed is the richest dietary source of the lignan, secoisolariciresinol (SECO), a diphenolic compound when ingested by humans influences a wide range of biological systems that keep humans healthy (Muir 2010). γ-Glutamyl transpeptidase levels are increased abnormally high during inflammation, in alcoholics with abscess and degenerative disease conditions including tumerogenesis. Flaxseed chutney diet showes significant reduction in both γ-glutamyl transpeptidase level

(52%) and micronuclei formation (47%) in rats (Shakir and Madhusudhan 2007). It is reported that the crude lipid contains 92.5% neutral lipids, 3.1% phospholipids, 2.4% acidic lipids and 2.1% free fatty acids. Oil extracted from flaxseed hull using supercritical CO_2 shows highest antioxidant capacity and the resultant defatted meal contains the highest (53 mg/g) secoisolariciresinol diglucoside which is an antioxidant phytochemical (Oomah and Sitter 2009). Prasad *et al*. (1998) studied the antiatherogenic activity of lignans present in flaxseed from the reduction in hypercholesterolemic atherosclerosis due to a decrease in serum total cholesterol and LDL-cholesterol. Addition of flaxseed into processed products is on the rise to extract the beneficial aspects of high α-linolenic acid content and the lignans present. Incorporation of flaxseed at 10, 15, and 20% to corn tortillas exhibited a high amount of total unsaturated fatty acids between 26.3 and 30.1% (Rendon *et al*. 2009). The α-linolenic acid content of raw and cooked beef patties increased as the level of flaxseed flour increased. The PUFA/SFA ratio in the control with 10% fat increased from 0.04 to 0.62 in the raw beef patties with 15% flaxseed flour. Addition of flaxseed and flaxseed oil in bread making has more effectively retained moisture and softness during 6 days storage at room temperature (Terhi *et al*. 2006). Flaxseed at 30–50% substitution for flour greatly enhanced the nutritional qualities of some nutrients without affecting the overall acceptability of bakery products. The detail chemical composition of flaxseeds is given in Table 2.1.

2.4.5 Niger seeds

Niger (*Guizotia abyssinica.)* is a member of the Asteraceae family. *Guizotia abyssinica* seed oil varies from 30 to 50% and is a good source of phytosterols which is in the amount of 4.0–4.2 g/kg of the total seed oil. β-Sitosterol, campesterol, stigmasterol, and D5-avenasterol comprise together about 90% of total sterols content, while β-sitosterol alone constitutes a half of the sterols content on supply of seed oils (Ramadan and Morsel, 2002).

The fatty acid profile shows a high content of linoleic acid (up to 63%) together with palmitic acid (17%), oleic acid (ca. 11%), and stearic acid (ca. 7%). The seed oil has 291 mg/g of neutral lipids, 5.76 mg/g of glycolipids, and 0.84 mg/g of phospholipids. Phospholipid classes consists of phosphatidylcholine (ca. 49%), phosphatidylethanolamine (22%), phosphatidylinositol (14%), phosphatidylserine (ca. 8%), and minor amounts (2–3%) of phosphatidylglycerol and lysophosphatidylcholine. Niger (*Guizotia abyssinica*)

seed could be nutritionally considered as a new non-conventio (Ramadan and Morsel, 2003).

Table 2.1 Chemical composition of oilseeds per 100g

Parameters	Perilla seeds	Niger seeds	Pumpkin seeds	Sesame seeds	Flax seeds
Moisture, g	7.4	4	8	7	5.4
Protein, g	17.4	24	24.3	19.1	24.2
Fat, g	51.7	39	47.2	48.2	40.4
Minerals, g	3.6	5	4.7	5.2	2.4
Fiber, g	2.1	11	0.2	3.6	9.2
Carbohydrate, g	18.2	17	15.6	17.9	18.4
Energy, Kcal	615	515	432	347	534
Calcium, mg	269	300	50	415	41.5
Phosphorus, mg	710	224	830	647	409.2
Iron, mg	9	57	5.5	-	1.5

(Source: Longvah and Deosthale, 1991)

In Nepal, niger is of some importance as a rain fed crop on marginal to poor soils, including sands and loams or well-drained clays. It is grown on approximately 5000–6000 ha and niger seed is Nepal's twelfth most important commodity export (Anon., 1999). The crop is usually grown in rotation with maize or rice on marginal sites in the hilly parts of the landscape and is used by rural people as edible oil, pickle, a cash crop or for burning oil in lamps. Its reported uses by industry are as an edible oil, a bird feed (especially in American markets), and in paint and soap making. In Nepal, Its production ranges at low altitude sites representing the central and eastern terai regions and the highest sites in the high mid hill regions. However, niger is reported to be rare in the western terai in Nepal, possibly due to the lower rainfall in the west. The niger crop in Nepal is sown usually in mid to late August, with some farmers sowing as early as late July and as late as early October. Harvest usually takes place in early December but this ranges from early November to late December (Mishra *et al.*, 2011). The detail chemical composition of perilla seed is given in Table 2.1.

2.4.6 Ginger

Ginger is the rhizome of *Zingiber officinale,* a perennial herbaceous plant native to south southern Asia. Ginger is cultivated in several parts of the world, the most important producing regions being India, China, Nigeria, Sierra, Leone, Indonesia, Bangladesh, Australia, Fiji, Jamaica and Nepal. The plant grows well in rich, sandy loam soil in a warm humid climate with good rainfall. Harvesting is carried out about ninth months after planting, when the leafy stems turn yellow and start withering. The ginger contains essential oil which is responsible for the aroma of ginger and gingerol responsible for its pungency (Lewis, 1984). The refreshing pleasant aroma, biting taste and carminative property of ginger make it an indispensable ingredient of food processing throughout the world. Fresh ginger, ginger powder from dry ginger, oleoresin and oil are all used for this purpose. It is an important ingredient of *ayuerveda* medicine and Chinese traditional medicine (Sivarajan and Balachandran, 1994).

Ginger has excellent antioxidant properties. Antioxidants are increasingly linked to the prevention of certain cancers and coronary heart disease, as well as their more established role in preserving lipid-based foods. Studies include the role of components such as gingerol in inhibiting linoleic acid, autoxidation, extending the shelf-life of meat dehydrated pork and fermented meat sausage (Kikuzaki and Nakatani, 1993). Ginger has beneficial effects on the digestive system, enhancing gastrointestinal motility, and is used traditionally for the treatment of stomach ache, vomiting and indigestion. It has also been investigated for its gastro-protectant and anti-ulcer activity (Yamahara *et al*., 1990). Ginger checks cholesterol biosynthesis and thereby inhibits hypercholesterolemia. Its role in Chinese herbal medicine in controlling obesity has been investigated (Wijaya and Wu, 1995). Ginger has a known influence on the eicosanoid cascade which influences such functions as wound healing, inflammation and platelet aggregation, and is involved in conditions such as arteriosclerosis (Srivasta, 1986). The chemical composition of ginger is given in Table 2.2.

2.4.7 Coriander

The name coriander (*Coriandrum sativum*) actually originated from the Greek word Koris meaning bedbug. It is an annual and herb of the apiaceae family, native to south- eastern Europe. It will tolerate wide range of climatic conditions and is now cultivated extensively throughout the world, including the temperate country of central and western Europe, the

mediterranean region, North and south America and India (Smith, 1982). It is an important spice crop and occupies a prime position in flavoring substances. The fruits are extensively employed as a condiment in the preparations of curry powder, pickling spices, sausages and seasonings. They are also used for flavoring pastry, biscuits, buns and cakes, and in flavoring liquors, particularly gin. Coriander seeds are also known for their medicinal properties and are considered carminative, diuretic, tonic, stomachic, refrigerant and aphrodisiac. As such, coriander is a frequent ingredient in the preparation of *ayurvedic* medicines and is a traditional home therapy for different ailments (Parthasarathy and Zachariah, 2008). Coriander has been advocated as an anti-diabetic remedy. It has been reported to have a number of possible medicinal attributes including antispasmodic, carminative and stomachic properties (Alison and Peter, 1999). Various studies demonstrate the presence of anti-hyperglycaemic, insulin releasing and insulin-like activity in coriander (Gray and Flatt, 1999). The chemical composition of coriander is given in Table 2.2.

2.4.8 Cumin

Cumin is one of the commonly used spices in food preparations. It is also used in traditional medicine as a stimulant, a carminative and an astringent. Cumin seeds are used as popular aromatic herbs and culinary spices. The seeds have a warm bitterish taste and a strong aromatic smell. Cumin seeds are used in curry powder or spice mixes. There are three varieties of cumin, viz., cumin (*Cuminum cyminum*), black cumin (*Nigella sativa*) and bitter cumin (*C. nigrum*). Cumin (*C. cyminum*) is a small annual, herbaceous plant belonging to Umbelliferae. It is cultivated in Arabia, India and China. The seeds are oblong in shape, thicker in the middle, compressed laterally, and have nine fine ridges about 5-in. long. Black cumin (*N. sativa*) is a member of the Ranunculaceae family (Hashim and Elkiey, 1962).

Cumin and black cumin are widely used as spice condiments in vegetarian and non-vegetarian preparations along with other spices in India and Arabia. Bitter cumin (Shahi jeera) is used in food preparations as a flavoring agent and pickles. All the cumin varieties are used in traditional and veterinary medicine as a stimulant, a carminative, an astringent, and as remedy against indigestion, flatulence and diarrhea (Norman, 1990).

Cuminaldehyde, cymene and terpenoids are the major constituents of volatile oils of cumin. Thymoquinone, dithymoquinone, thymohydroquinone and thymol are the

pharmacologically active quinones of black cumin oil. Bitter cumin is rich in monoterpene aldehydes. (Thippeswamy and Akhilender, 2005). Cumin decreased significantly the plasma levels of cholesterol, triglycerides and phospholipids and activity of the enzymes aspartate transaminase, alkaline phosphatase and gamma glutamyl transferase (enzymes that are non-specific indicators of tissue damage such as liver disease (alcoholic liver disease, chronic hepatitis, cirrhosis, obstructive jaundice, hepatic cancer), myocardial infarction, pancreatitis and muscle-wasting diseases) when compared with the normal control group (Aruna *et al*., 2005). Cumin can be used to stimulate the appetite and relieve dyspepsia and diarrhea. It may stimulate the secretion of pancreatic enzymes, which could explain its effect on the digestive system (Amin, 2000). It is emmenagogic and antisplasmodic. It is believed to increase lactation and reduce nausea in pregnancy (Weiss, 2002). The chemical composition of cumin is given in Table 2.2.

2.4.9 Red chilli

Red chillies (*Capsicum annum*) belongs to the family Solanaceae. Plants are bushy, about 60–80 cm high and are semi-perennials that are grown as annuals in cultivation. Red chillies are extensively used in the form of powder, flakes, paste and whole chilli. Chillies are one of the largest traded spice in the international market. Red chillies are also known as Capsicum, Paprika etc. depending on the species and variety. This spice is extensively used for food flavoring and coloring and also for certain medicinal properties. The principal coloring matter is the carotenoid pigment 'capsanthin' constituting about 35% of the total pigments. The demand for natural pigments of red chillies is increasing because of their use as organic food colors and so offer good potential for export (Prabhavathi *et al.,* 2008).

2.4.10 Turmeric

Turmeric comes from the root of the *Curcuma longa* plant and has a tough brown skin and a deep orange flesh. It is a tropical plant that is cultivated extensively in Asia, India, China, and other countries with a suitable climate. *C. longa*, is a perennial herb, and a member of the ginger family. It can grow up to 1 m high, and has oblong, tufted leaves. The yellow spice is made from the rhizomes, which are boiled, dried, and then ground. The active component in turmeric is curcumin, which may constitute 2 to 8% of the spice. Curcumin is a non-water-soluble polyphenol that can be derived from turmeric by ethanol extraction

(Dobelis, 1986). It has traditionally been used as a coloring agent in Asian cuisine, as well as in cheese, butter, yogurt, and other kinds of food (Arun *et al*., 2002).

Curcumin has been used throughout history, especially in India and Asia. It is used for several purposes apart from flavoring and coloring food. Numerous studies have shown that curcumin has antioxidant and anti-inflammatory properties (Hsu and Cheng, 2007). Recent studies have also indicated that curcumin affects cellular enzymes, and angiogenesis (Chainani, 2003). It has protective role against diabetic nephropathy (Sharma *et al*., 2006).

2.4.11 Salt

Salt has been used both to flavour and preserve food since the earliest recorded times. The word "salt" as commonly used and in the sense used here denotes a single substance, in chemical nomenclature "sodium chloride". It is a compound of sodium ions (positively charged sodium atoms) and chloride ions (negative charged chlorine atoms). They are present in equal numbers but the proportion of chloride to sodium by weight is roughly 60:40. Both are essential nutrients but sodium has attracted more attention. Sodium ions fulfil several indispensable body functions. They are not only present in and outside all the cells of body, they must be present in the right concentrations for each part of the body function. They are necessary for the transfer of molecules (e.g. amino acids) across membranes, for the transmission of nerve impulses, for the digestion of food and for muscular action (Heimbach, 1986). The maximum recommendation of salt for adult is 6g per day (WHO, 1990).

As a way of preserving food, salt works primarily by rendering any water present unavailable for the growth of micro-organisms. The effect on taste and flavor, however desirable, is incidental to this. When salt is used for flavoring, it not only adds its own taste, but modifies and intensifies the intrinsic flavor of the food (Anon., 1984).

2.4.12 Acid (*Chuk amilo*)

The lemon is a citrus fruit. Lemon juice consists for 5% of citric acid, C6H8O7, and contains other substances such as vitamine C. Lemons contain unique flavonoid compounds that have antioxidant and anti-cancer properties. Of special interest in lemons are flavonoids called flavonol, glycosides, including many other molecules. While these flavonoids have been shown to stop cell division in many cancer cell lines, they are

perhaps most interesting for their antibiotic effects. Some bitterness to the juice is due to limonoids (Rangana *et.al.*, 2009).

The Lemon Juice Concentrate is a natural product obtained by partly evaporating the water from the freshly squeezed juice of naturally matured sound and clean lemons. The juice is pasteurised and then immediately concentrated in order to guarantee its perfect preservation. It contains no artificial sweeteners or other additives.

(http://www.juiceworld.net/Concentrates/lemon_juice_concentrate.htm)

Table 2.2 Chemical composition of spices per 100 g

Parameters	Ginger	Cumin	Coriander	Turmeric	Red chilli
Moisture, g	9.38	11.9	8.86	11.36	8.05
Energy, Kcal	347	356	298	354	318
Protein, g	9.12	18.7	12.37	7.83	12.01
Fat, g	5.95	15	17.77	9.58	12.27
Carbohydrate, g	70.79	36.6	54.99	64.93	56.63
Ash, g	4.77	5.8	6.02	6.02	6.04
Calcium, g	0.116	0.8	0.709	0.182	0.148
Phosphorus, mg	148	511	409	268	293
Sodium, mg	32	126	35	38	130
Potassium, mg	1342	980	1267	2525	2014
Iron, mg	11.52	11.7	16.32	41.42	7.8
Thiamine, mg	0.046	0.55	0.239	0.152	0.328
Riboflavin, mg	0.0185	0.36	0.29	0.238	0.919
Niacin, mg	5.155	2.6	2.132	5.14	8.701

(Source: USDA Agricultural Handbook, 1997)

2.5 Composition of *dhule achar*

Dhule achar is made up of ginger, turmeric, chilli, cumin, coriander, salt and acid mixed with roasted oilseeds powder. The oilseeds may be perilla seed, niger seed, pumpkin seed, flaxseed and sesame seed. Roasted oilseed powder constitute of nearly 70%, salt constitute

of 5% and total of other spices constitute 19% of the weight of *dhule achar*. *Chuk amilo* (lemon juice concentrate) is used and the acidity of the product is maintained to be 1.5 %. The ingredients used in *dhule achar* are rich in fat and protein. The earlier studies of Nwokolo and Sim, (1987) reported that pumpkin seed kernel flour was superior to soybean in its content of all amino acids except lysine. Protein is a source of amino-acids and nitrogen, needed for the synthesis of the cell growth. Adequate protein intake is particularly important during period of growth or recovery from the disease (Guthrie and Picciano, 1989).

Fat content of *dhule achar* is contributed by the spices and oilseeds. Fat content vary with oilseeds used. So, *dhule achar* is a fatty food depending on the types of oilseeds used for its preparation. Further, the essential oil of spices is rich in natural antioxidants. According to Guthrie and Picciano (1989), dietary fat is efficient source of calories. Fat is protein sparing that is fat is burned for energy saving valuable proteins for their important roles.

Similarly, *dhule achar* is mineral rich food, a large proportion of it contributed by the spices and oilseeds. Spices like ginger, turmeric, and cumin used in *dhule achar* preparation are very rich source of iron. According to Robert, (2006), iron is very essential to the growing children and pregnant women. Iron deficiency in pregnant women is correlated to infant prematurity, low birth weight, weak immune system, growth failure and high maternal mortality rate.

Food composition table of the ingredients of *dhule achar* shows that it is rich in minerals like sodium, potassium, phosphorous and vitamins like riboflavin, and niacin. According to swaminathan (2003), sodium is the major extracellular electrolyte. Sodium ion functions as a participant in the regulation of osmotic pressure, role in nerve conduction, active transport, and water balance. Sodium ion plays an important role in the absorption of monosacharides and amino acids from the small intestine. Also, it plays role in initiating and maintaining the heart beat. According to Underwood and Suttle (1999), potassium is a major determinant of intracellular osmolality. Potassium is needed for cell membrane polarization, which influences process like nerve impulse and muscle cell contraction.

Further spices used in *dhule achar* have some beneficial physiological effect on human health. Spices like ginger, cumin, coriander have beneficial effects on the digestive system,

stimulates the taste buds, enhances gastrointestinal motility and prevents from flatulence and indigestion. They also have antimicrobial properties and wound healing properties (Sivarajan and Balachandran, 1994).

Since there is variation in the spices and oilseeds source for the preparation of *dhule achar*, its composition may vary accordingly.

2.6 Storage stability of fatty foods

The word stability refers to the capability of fatty foods to maintain a fresh taste and odour during storage. It is related to composition of lipid moiety, the presence or absence of antioxidants, effectiveness of packaging, temperature, moisture, air, light etc (Holley and Tember, 1983). In fatty foods, stability always refers to the chemical rather than physical alteration. The chemical changes which occur in fatty foods, can be divided into two main classes, oxidative and hydrolytic deterioration known as rancidity (Heid and Joslyn, 1975).

Autoxidation is rather a complex free radical reactions. Fatty acids hydroperoxides formed during this reaction sequence are then decomposed by a series of dissimilation reactions. Severals of products formed are radicals and undergo further radicals reactions. Other such as hydroxyl acids, keto acids and aldehyde are commonly found in oxidised lipid system. Many of the short chain volatile compounds are responsible for the off-flavors and odours characteristics to the rancid foods (Shmilt, 1982). Some fatty foods have a tendency to develop an off flavor known as flavor reversion. Fatty foods containing linolenic acid show this phenomenon, when exposed to the air (Deman, 1976).

2.6.1 Factors affecting the rancidity

2.6.1.1 Light

Visible light seems to split up the decomposition of hydroperoxides. This may be the results of absorption of the light by the peroxides or other compound that may be present. The action of ultraviolet light has a greater effect. When the polyunsaturated fatty acids are autoxidized, the result is the formation of conjugated unsaturated systems. These in-turn absorb the ultraviolet strongly at certain wavelength under these conditions the ultraviolet materially speeds the breakdown of peroxides. Peroxides are the primary products of oxidation, and hence measurement of their concentration gives an idea about the extent about oxidation (Joshi, 1994).

2.6.1.2 Heavy metals

Heavy metals Fe, Cu, Co, Mn, Ni increase the maximum rate of oxidation. The metal ions initiate free radical chain by the electron transfer. So, presences of traces of heavy metal in lipid foods without doubt one of the important reason for their oxidative deterioration (Lee, 1983).

2.6.1.3 Temperature

The rate of reaction is accelerated by increasing temperature. The energy of activation is strongly depend upon the temperature. In lipid system temperature accelerate both the generation of free radicals and their disappearances (Bockisch, 1998).

2.6.1.4 Oxygen

As long as the oxygen is present in limited quantity, the rate of autoxidation increases with increasing oxygen pressure until a constant oxidation rate is reached beyond the given pressure (Berk, 1976). Min and Wen (1983), reported the formation of volatile compounds in fatty foods during storage can be minimized by lowering the oxygen content.

2.6.1.5 Presence or absence of antioxidants

Any substances which is capable of delaying retarding or preventing the development of rancidity or other flavor deterioration due to oxidation is termed as antioxidant (Allen and Hamilton, 1983).

Many naturally occurring substances function as antioxidants. Most prominent are the tocopherols (α, β, γ, δ). This comound acts as biological antioxidants in plant and animal tissues and residual quantities of tocopherols aid in maintaining keeping quality of lipid rich food. The remarkable stability of sesame seed oil is due to the presence of sesamin, sesamolin, sesaminol, sesamol, and α-tocopherol acting as antioxidant (Yamashita *et al*., 2000).

Many compounds have been developed synthetically for maintaining the keeping quality of fats and oils. Such compounds include butylated hydroxyl tolune (BHT), butylated hydroxyl anisole (BHA), Propyl gallate (PG), tertiary butylhydroquinone (TBHQ) etc. (Wanasundara *et al*., 2008).

2.6.1.6 Moisture

The quantity of moisture present in the product is one of the most important factor influencing the storage life. Hydrolytic rancidity is generally caused by the combination of microorganism and moisture. This factor tends to reduce the quality of the product whether by microbial growth or by accelerating the chemical reaction (Hall, 1980). The rancidity develops more rapidly both at very high moisture and very low moisture levels. Maximum stability is observed at intermediate moisture levels, corresponding to monolayer values (Berk, 1976).

2.6.1.7 Degree of unsaturation

The polyunsaturated fatty acids and other highly unsaturated fatty compounds are particularly susceptible to oxidative rancidity. So presence of unsaturated fatty compounds influences the rancidity.

2.6.2 Prevention from rancidity

One of the major problems encountered in the storage of foodstuffs is autoxidation of their lipid components. Oxidation limits the shelf life of many products including meats, edible oils, oilseeds product and cereal-based foods (Salih *et al*., 1989). In living organisms, oxidation is prevented by a number of endogenous systems such as enzymes, antioxidants, and chelators of metal ions (Halliwell *et al*., 1999). Once removed from the organism, lipids may be protected from oxidation in several ways (He and Shahidi, 1997). These include the use of low-temperature storage to slow down the oxidation process, the use of advanced packaging technologies to exclude oxygen and light, and the addition of antioxidants. Many of the antioxidants in common use are of synthetic origin (Winata and Lorenz, 1996); these include BHA, BHT, TBHQ, and propyl gallate as well as both synthetic and natural tocopherols. Unfortunately, despite their effectiveness, synthetic antioxidants have raised health concerns because both BHA and BHT are suspected carcinogens (Madsen and Bertelsen, 1995). Thus, there is an interest in using natural antioxidants to protect food lipids from oxidation.

2.6.3 Importance of peroxide value and acid value in accessing food quality and food safty

Fats and oils in foods are oxidised during processing, circulation and preservation, this reaction causes deterioration in taste, flavor, odour, color, texture and appearance, and decrease in the nutritional value of the food. Furthermore, the reaction can induce food poisoning. From a food quality and food safty prospective, this oxidation reaction must be suppressed. *Dhule achar* is a lipid rich food and therefore liable to oxidation unless properly stored. Australian Oilseeds Federation (AOF) has set standards for oilseeds in the food sanitation law to protect against food poisoning and to control the quality of oil and oilseeds product. According to this standard, the maximum peroxide value for oilseeds and vegetable oil is 10 meq/kg. Acid value is an index to measure the free fatty acid. The FFA themselves are not toxic; however , the presence of FFA affects food quality. Consequently, measuring both indices is indispensible to control food quality and safty (Lundberg, 1961). At the stage of fat and oil deterioration, the reason for measuring PV and AV are very different because of the different mechanism underlying the formation of hydroperoxides and FFA from fat and oil. Hydroperoxides are formed by the oxidation of fats and oil. Whereas FFA is formed by the hydrolysis of fat and oil. PV is an index quantity the amount of hydroperoxides in fat and oil. Several studies have reported those secondary oxidised oil products are generally toxic (Artman, 1969). Oxidised fats and oil induce neurotoxin relating Pica behavior (the indiscriminate craving for and eating of substances such as paints, chips, clay, plaster or dirt) and Hypoactivity. Therefore, the formation of hydroperoxides the primary oxidised products of fat and oil must be suppressed to protect against the oxidation of fat and oil and the formation of secondary oxidised products from both food quality and food safty perspectives (Lundberg, 1961).

Part III

Materials and methods

3.1 Materials

3.1.1 Material collection

The raw materials used in the preparation of *dhule achar* were turmeric, ginger, chilli, coriander, cumin, salt, acid and oilseeds (sesame seeds, niger seeds, perilla seeds, flaxseeds and pumpkin seeds). All the raw materials were collected from the local market of Dharan.

3.1.2 Chemicals, glasswares and equipments

Following chemicals, glassware and equipments were used for the present work as available from the laboratory of CCT, Dharan.

1. **Equipments:** Electronic balance, Hot air oven, Soxhlet assembly, Buchner filter assembly, Kjeldahl digestion and distillation set, Suction pump, Spectrophotometer, Flame photometer, dessicator, Muffle furnace, electric grinder, standard test sieve.
2. **Glasswares:** Silica crucible, pipette, Petri dish test tubes, burette, and other accessories.
3. **Chemicals:** Acetone, Bromocresol green, Phenopthalein indicator,Boric acid, Digestion mixture (catalyst mixture), Petroleum ether, Methyl red indicator, Methyl orange indicator, Potassium permanganate, Potassium thiocyanide, Sat. Pot. Persulfate, Reagent grade Ferrous amm. Sulfate. $6H_2O$, Analytical grade pot. Chloride., Conc. HCl, Conc. H_2SO, Conc. Ammonia, Acetic acid, $AgNO_3$.

3.2 Methods

3.2.1 Survey

A survey was conducted about *dhule achar* among 114 persons of different parts of eastern Nepal especially female who know about the preparation method of *dhule achar*. The survey was based on the questionnaire as given in the appendix A.

3.2.2 Processing of raw materials

3.2.2.1 Oilseeds

Oilseeds (sesame, niger, perilla, linseeds and pumpkin seeds) were cleaned separately to remove impurities such as stones, weed seeds, other grains etc. by using *nanglo*. Then they were roasted in iron dish under the flame of gas stove till they become brittle. The roasted oilseeds were then ground in electric grinder (Piccaso) into fine powder. The powder was sieved with laboratory sieve of 600 micron (SETHI Standard Test Sieve). Each of roasted oilseeds powder was kept in polythene bag before blending.

3.2.2.2 Spices

Spices like cumin, dry ginger, coriander, turmeric and chilli powder were individually sun dried for 5 hours. The dried spices were then ground in electric grinder (Piccaso) into powder. Each of the spices powder was sieved with laboratory sieve of 300 microns (SETHI Standard Test Sieve) and kept in polythene bag before blending.

3.2.2.3 Salt

Salt was ground in electric grinder of laboratory. Then, salt powder was sieved with laboratory sieve of 300 microns (SETHI Standard Test Sieve) and kept in polythene bag before blending.

3.2.2.4 *Chuk amilo*

Concentrated citrous juice locally known as *Chuk amilo* was used as acid. It was bought from local market which contained 25% acidity (as citric acid).

3.2.3 Recipe formulation

Recipe for the *dhule achar* preparation was formulated based on the survey conducted in different parts of Nepal as per the questionnaire prepared given in Appendix A. The recipe given by most people was chosen for the *dhule achar* formulation in this work.

3.2.4 Product formulation

The product formulation of *dhule achar* was carried out in two stages. At first, six formulations were made in which five formulations accounted individual oilseeds and one formulation accounted equal proportion of each oilseeds. The acid and spices (cumin, coriander, turmeric, chilli, ginger and salt) were kept same in different formulations. Spices

mixture was prepared by blending individually roasted and grinded spices items (cumin, coriander, turmeric, chilli, ginger and salt). *Chuk amilo* was used as acid as shown in Table 3.1

Table 3.1 Different product preparation of *dhule achar*

Formulation	Ingredients
A	Roasted niger seed powder + acid + spices mixture + salt
B	Roasted flax seed powder + acid +spices mixture + salt
C	Roasted (sesame + perilla + niger + flaxseed + Pumpkin seed) powder in equal proportion + acid + spices mixture + salt
D	Roasted pumpkin seed powder + acid + spices mixture + salt
E	Roasted perilla powder + acid + spices mixture + salt
F	Roasted sesame powder + acid + spices mixture + salt

In 2nd stage, the product formulation was developed by using combination of all five oilseeds in different proportion. The acid and spices (cumin, coriander, ginger, chilli, turmeric and salt) were also kept constant here. The formulation is as shown in Table 3.2.

Table 3.2 Product formulation of *dhule achar*

Formulation	Ingredients
A	roasted 30% sesame + each 10% (perilla + flax + pumpkin seed + niger) powder + acid + spices mixture + salt
B	roasted 30% perilla + each 10% (pumpkin seed + flax + niger + sesame) powder + acid + spices mixture + salt
C	roasted 30% flaxseed + each 10% (niger + sesame + flaxseed + pumpkin seed) powder + acid + spices mixture + salt
D	roasted 30% pumpkin seed + each 10% (flax + niger + Sesame + perilla) powder+ acid +spices mixture + salt
E	roasted 30% niger + each 10% (sesame + pumpkin seed + perilla + flaxseed) powder + acid + spices mixture + salt

3.2.5 Product preparation

Each ingredient's powder was weighed as per the formulation. Then the ingredients were mixed uniformly by keeping them in mixer.

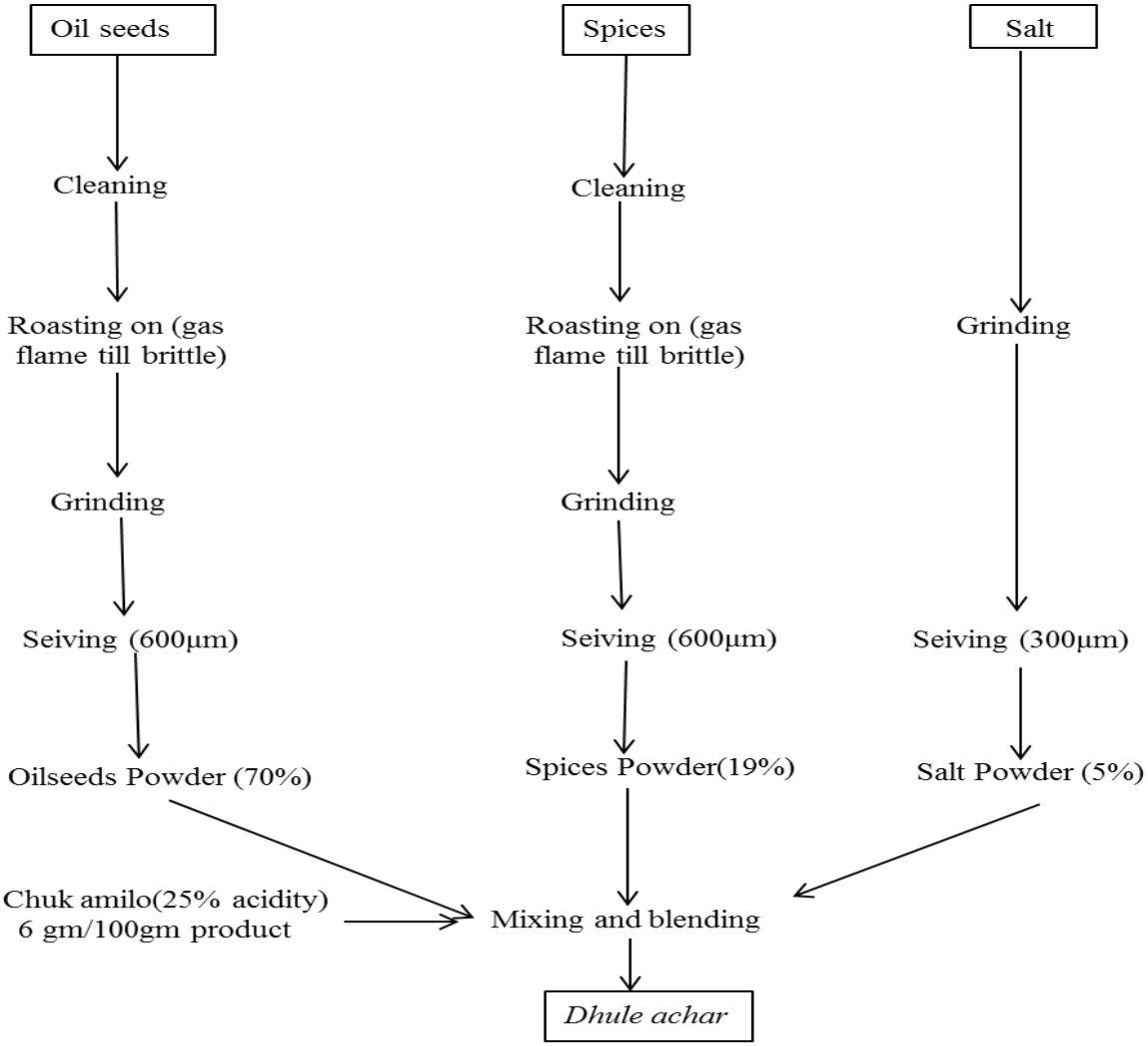

Fig. 3.1 Process outline for *dhule achar*

The uniformly mixed product is then packed in polypropylene pack of 80 µm thickness. Spices include turmeric, ginger, coriander, cumin, chilli, and salt. Common iodine salt and *chuk amilo* were added to the product. The oilseeds were sesame seeds, pumpkin seeds, perilla seeds, niger seeds and flax seeds. Detail outline of product preparation is given in Fig. 3.1.

3.2.6 Analysis of chemical composition of product

3.2.6.1 Proximate composition

- **Moisture content**

Moisture content was determined using hot air oven (Ambassador, working temperature 0 to 300^{o}C, UK) as per Egan *et al.* (1981).

- **Crude protein**

Crude protein (N × 6.25) was determined by micro kjeldahl method as per Egan *et al.* (1981).

- **Crude fat**

Crude fat content in the product was determined by soxhlet extraction method as per Ranganna (2009).

- **Total ash**

Total ash was determined by ashing in electric muffle furnace (Ambassador, working temperature, 500°C, UK) as per Ranganna (2009).

- **Crude fiber**

Crude fiber was determined as per AOAC (2005).

- **Total carbohydrate**

Total carbohydrate was determined by difference.

Total carbohydrate (%) =100- (moisture + crude protein + crude fat + crude fiber + ash) %

3.2.6.2 Mineral analysis

- **Iron**

Iron content was determined by colorimetric method as per Ranganna (2009).

- **Calcium**

Calcium content was determined by precipitation method as per Egan *et al.* (1981)

- **Sodium**

Sodium was determined by flame photometry method as per Rangana (2009).

- **Potassium**

Potassium was determined by flame photometry method as per Rangana, (2009).

3.2.7 Estimation of peroxide value

The product was analysed for Peroxide Value at interval of 15 days as per AOAC (2005).

3.2.8 Estimation of acid value

Acid value was determined by titration method at interval of 15 days as per AOAC (2005).

3.2.9 Sensory analysis

Sensory evaluation was performed by 9 point hedonic scoring test (9 = like extremely, 1 = dislike extremely) for color, flavor, texture, taste and overall acceptance. Different formulations were prepared by varying their proportions and the products were served in identical odor free disposable plastic containers. The evaluation was carried out by 20 panelists comprising of teachers and students of CCT including 4 female and 16 male. Sensory evaluation was carried out in individual booth with adequate light and free from obnoxious odors. Each panelist was provided with 6 samples in first step and 5 samples in second step coded with three digits random numbers and evaluation card (Appendix B). They were provided with portable water for rinsing between the samples. Verbal communication among the panelist was prohibited. They were asked to evaluate the samples individually using score card.

3.2.10 Data analysis

Data on sensory analysis were tabulated for comparison and were graphically represented using Microsoft Excel-2002(10.2614.2625) Copyright Microsoft Corporation 1985-2001. Data were statistically processed by GenStat Discovery Edition 3, GenStat Procedure Library Release PL15.2, Version 7.22 DE (Copyright 2008, VSN International Ltd) for Analysis of Variance (ANOVA). Means of the data were separated whether they are significant or not by using LSD (least square difference) method at 5% level of significance.

Part IV

Results and discussion

4.1 Survey result

A survey study was designed to collect the required information about *dhule achar*. A questionnaire was developed (Appendix A) and answers given by the surveyed people were filled up. The surveyed people belonged to different geographical locations, religion and ethnicity, age and sex. The survey was conducted among total of 114 respondents mainly in the different districts of Eastern region (Terhathum, Dhankuta, Sankhuwasava, Sunsari, Morang, Jhapa) and Central region of Nepal (Kathmandu, Makawanpur, Palpa, Chitawan). The highest percentage (67%) of the surveyed people comprised of age between 40 and 80 years followed by 20-40 years. From the survey following information about the *dhule achar* were collected. *Dhule achar* is the traditional, nutritious and medicinal powdery pickle indigenous to Nepal. Almost all (95%) of the surveyed population said that *dhule achar* was originated since the time immemorial and they had obtained the skill of its preparation from their ancestors. From survey, its ingredients include five items of oilseeds (sesame, perilla , niger, flax and pumpkin seed), acid (*chuk amilo*) and five items of spices (cumin, coriander, ginger, turmeric and chilli powder) along with salt. However, slight variation was found regarding the use of oilseeds and spices. Majority, (71%) of the surveyed people mentioned the use of pumpkin seed and niger seed. Regarding the use of oilseeds, 60% of the surveyed people mentioned the use of individual oilseeds while 40% people mentioned the use of these oilseeds in certain proportion for the preparation of *dhule achar*. Some of the surveyed people (26%) also mentioned the use of black soyabean in *dhule achar* preparation. Regarding the use of spices, about 70% of the surveyed population mentioned the use of cumin, coriander, turmeric, ginger and salt while, very few of them mentioned the use of only cumin, ginger and salt. The most likely preferred acid by the surveyed people was lemon juice concentrate (*chuk amilo*). Almost all (83%) of the surveyed people mentioned the similar preparation method of *dhule achar*. The household preparation method is simple. Firstly, each ingredient is cleaned separately using *nanglo*. Spices are sun dried to make it friable and readily millable and then grinded in okhali. Similarly, oilseeds are roasted and then grinded in *okhali* and then sieved to separate the large size particles. The ingredient's

powder is then mixed. Around 30% of the surveyed populations mentioned its use only in feasts and festivals such as *kajkriya, bratabandha, puja, marriage ceremony* etc. 70% of the surveyed people mentioned its preparation at any time if the raw materials are available. Because of its potent appetizing and digestive property, it is preferred by almost all community in Nepal. Most of the surveyed people (87%) preferred to consume *dhule achar* along with rice and breakfast items such as bread etc. The survey report shows that *dhule achar* is still not sold commercially in market and its use is confined to the household level.

4.2 Preparation of *dhule achar* from varieties of oilseeds

The recipe for the preparation of *dhule achar* was obtained from survey conducted in different parts of Nepal. The recipe given by most people during the survey was used in the work which is given in Table 4.1

Table 4.1 Recipe formulation of *dhule achar* per 100 g product.

Ingredients in (gm)	Formulation					
	A	B	C	D	E	F
Niger seeds	70	-	14	-	-	-
Pumpkin seeds	-	70	14	-	-	-
Perilla seeds	-	-	14	-	70	-
Flax seeds	-	-	14	70	-	-
Sesame seeds	-	-	14	-	-	70
Cumin	4	4	4	4	4	4
Ginger	4	4	4	4	4	4
Coriander	4	4	4	4	4	4
Turmeric	3	3	3	3	3	3
Red chilli	4	4	4	4	4	4
Salt	5	5	5	5	5	5
Chuk amilo	6 g	6 g	6 g	6 g	6 g	6 g,

The recipe is in accordance with the amount mentioned by majority (75%) of the surveyed people. The variation in amount of spices wasn't carried out. Thus, in first stage, five samples of *dhule achar* were prepared from individual oilseeds and a sample of *achar* was prepared from equal proportion of each oilseeds. As the people traditionally mix the

ingredients without exactly weighing based on their assumption and experience so most of the people mentioned the amount of ingredients in round figure. During the survey, different people mentioned the use of different oilseeds depending on the availability and their liking. So, variation in the oilseeds was taken as the basis for product formulation.

4.3 Effect of oilseed varieties on sensory quality

At first stage, six items of *dhule achar* were prepared, five from respective oilseeds and one from equal proportion of each oilseed.

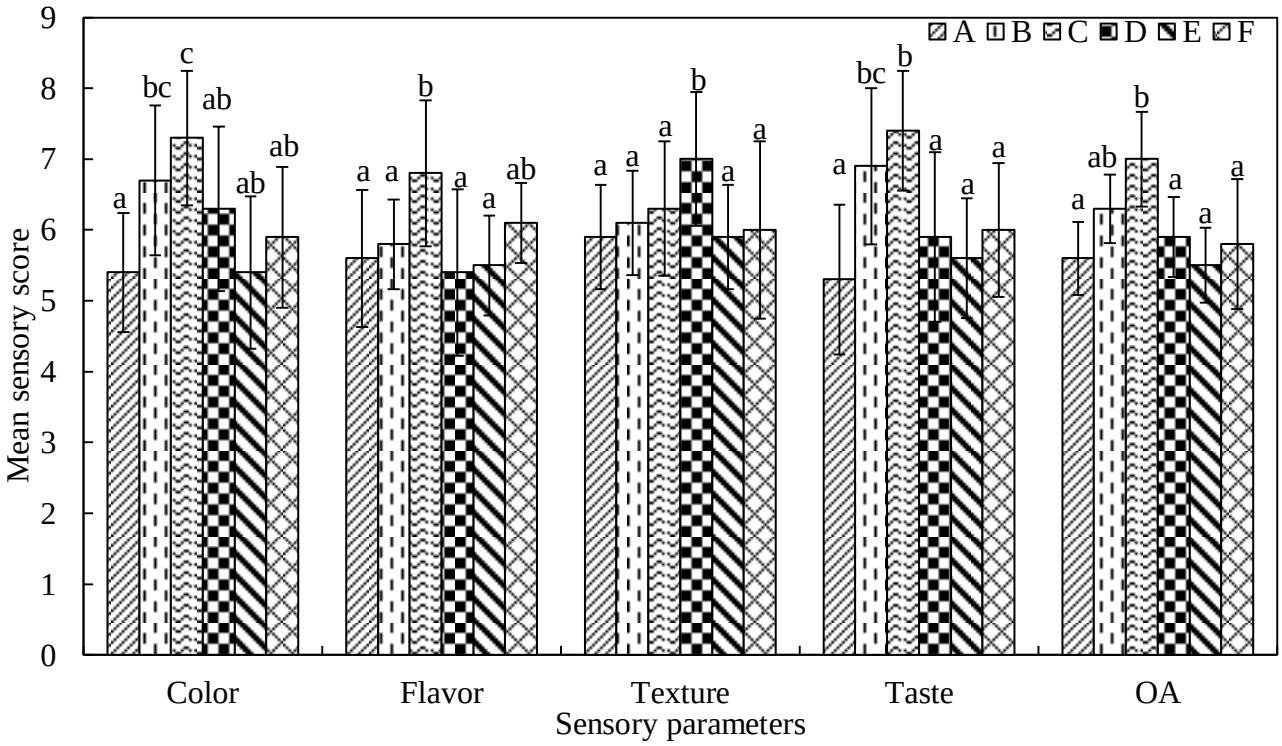

Fig. 4.1 Effect of oilseed varieties on sensory quality of *dhule achar*

[Note: A= Roasted niger seeds powder + acid +spices mixture + salt , B= Roasted flax seeds powder + acid + spices mixture + salt, C= Roasted (sesame + perilla + niger seeds+ flax + Pumpkin seeds) powder in equal proportion + acid + spices mixture + salt, D= Roasted pumpkin seeds powder + acid + spices mixture + salt, E= Roasted perilla seeds powder + acid +spices mixture + salt, F= Roasted sesame seeds powder + acid +spices mixture + salt and OA represent overall acceptance

Similar alphabet above the bar indicates not significantly different ($p>0.05$). The error bars show the standard deviation.]

4.3.1 Color

The mean sensory score for color were 5.4±0.84, 6.7±1.06, 7.3±0.95, 6.3±1.16, 5.4±1.07 and 5.9±0.99 respectively for sample A, B, C, D, E and F. The obtained mean values are represented as bar diagram in Fig. 4.1. ANOVA of mean showed that there is significant difference in color within the sample ($p \leq 0.05$).

LSD testing showed that the product preparation had effect on color of *dhule achar*. Sample C had highest score (7.3±0.95) while sample A (5.4±0.84) had lowest. There was no significant difference among the samples A, D, E and F; B, D, E and F and B and C. Sample C was significantly different from all samples except sample B. Samples A and B; A and C; B and E; C and D; C and E and C and F were significantly different ($p \leq 0.05$) among themselves. The color of sample C which was made by equal proportion of all oilseeds was most liked. Creamy yellowish color of pumpkin seeds was moderately liked while brownish black color of niger and perilla seeds were least liked by majority of the panelists.

4.3.2 Flavor

The mean sensory score for flavor were 5.6±0.97, 5.8±0.63, 6.8±1.03, 5.4±1.17, 5.5±0.71 and 6.1±0.57 for samples A, B, C, D, E and F respectively. The obtained mean values are represented as bar diagram in Fig. 4.2. ANOVA of mean showed that there is significant difference ($p \leq 0.05$) in flavor within the samples.

Sample C (6.8±1.03) had the highest score while sample D (5.4±1.77) had the lowest. LSD showed no significant difference ($p \leq 0.05$) between the samples A, B, D, E, F and C and F. Sample C was significantly different from all samples except F. Flavor in food is the interaction of different constituents present in food. Pyrolysis is the roasting of foods to create flavor changes that make a food that is more desirable to consumers. Flavor precursors are lipids, sugars, proteins and amino acids, most typically containing aspartic, glutamic acids, asparagines-glutamine, phenylalanine and histidine (Buckholz and Daun, 1981).

4.3.3 Texture

The mean sensory score for texture were 5.9±0.74, 6.1±0.74, 6.3±0.95, 7±0.94, 5.9±0.74 and 6±1.25 for samples A, B, C, D, E and F respectively. The obtained mean values are

represented as bar diagram in Fig. 4.2. ANOVA of mean showed that there is significant difference ($p \leq 0.05$) in texture within the samples.

Sample C (6.3±0.95) had the highest score while sample A (5.9±0.74) and E (5.9±0.74) had the lowest. LSD showed that sample D was significantly different ($p \leq 0.05$) from all samples while samples A, B, C, E and F were not significantly different themselves. Sample C was made by equal proportion of all oilseeds and therefore it had improved flowability and soft consistency. So it may be the reason why most panelists liked this sample.

4.3.4 Taste

The mean sensory score for taste were 5.3±1.06, 6.9±1.10, 7.4±0.84, 5.9±1.19, 5.6±0.84 and 6±0.94 for samples A, B, C, D, E and F respectively. The obtained mean values are represented as bar diagram in Fig. 4.2. ANOVA of mean showed that there is significant difference ($p \leq 0.05$) in taste within the samples.

Sample C (7.4±0.84) had the highest score while sample A (5.3±1.06) had the lowest. LSD showed the samples B and C were significantly different from A, D, E and F. But the samples A, D, E and F were not significantly different themselves. This might be due to the sum effect taste of all ingredients in sample C.

4.3.5 Overall acceptance

The mean sensory score for OA were 5.6±0.52, 6.3±0.48, 7±0.67, 5.9±0.57, 5.5±0.53 and 5.8±0.92 for samples A, B, C, D, E and F respectively. The obtained mean values are represented as bar diagram in Fig. 4.2. ANOVA of mean showed that there is significant difference ($p \leq 0.05$) in taste within the samples.

Sample C (7±0.67) had the highest score while sample E (5.5±0.53) had the lowest. LSD test showed the sample C was significantly different ($p \leq 0.05$) from all samples except B. Samples A, B, D, E and F were also significantly different ($p \leq 0.05$) themselves.

From sensory analysis based on mean score on six attributes, sample C was chosen for the best formulation. The sample C was prepared by mixing equal proportion of all roasted oilseeds powder. So in second stage, five different formulations by varying the proportion of oilseeds were prepared and carried out for sensory analysis.

4.4 Preparation of *dhule achar* by variation in proportion of oilseeds

In second stage, *dhule achar* was prepared by varying the proportion of these five oilseeds. In this case, the amount of spices and acid wasn't varied. The recipe of the formulation is shown in Table 4.2.

Table 4.2 Recipe formulation of *dhule achar* per 100 g product

Ingredients in (gm)	Formulation				
	A	B	C	D	E
Sesame seeds	30	10	10	10	10
Perilla seeds	10	30	10	10	10
Flax seeds	10	10	30	10	10
Pumpkin seeds	10	10	10	30	10
Niger seeds	10	10	10	10	30
Cumin	4	4	4	4	4
Ginger	4	4	4	4	4
Coriander	4	4	4	4	4
Turmeric	3	3	3	3	3
Red chilli	4	4	4	4	4
Salt	5	5	5	5	5
Chuk amilo	6 g	6 g	6 g	6 g	6 g

4.5 Effect of proportion variation of oilseeds on sensory quality

From first sensory analysis it was known that mixed *dhule achar* was preferred rather than prepared from individual oilseeds. So in second stage five mixed *dhule achar* were prepared by varying proportion of oilseeds. It was done by taking one oilseeds as predominant and keeping other proportion of oilseeds constant. Spices mixture (cumin, coriander, turmeric, chilli, ginger), salt and *chuk amilo* were kept constant.

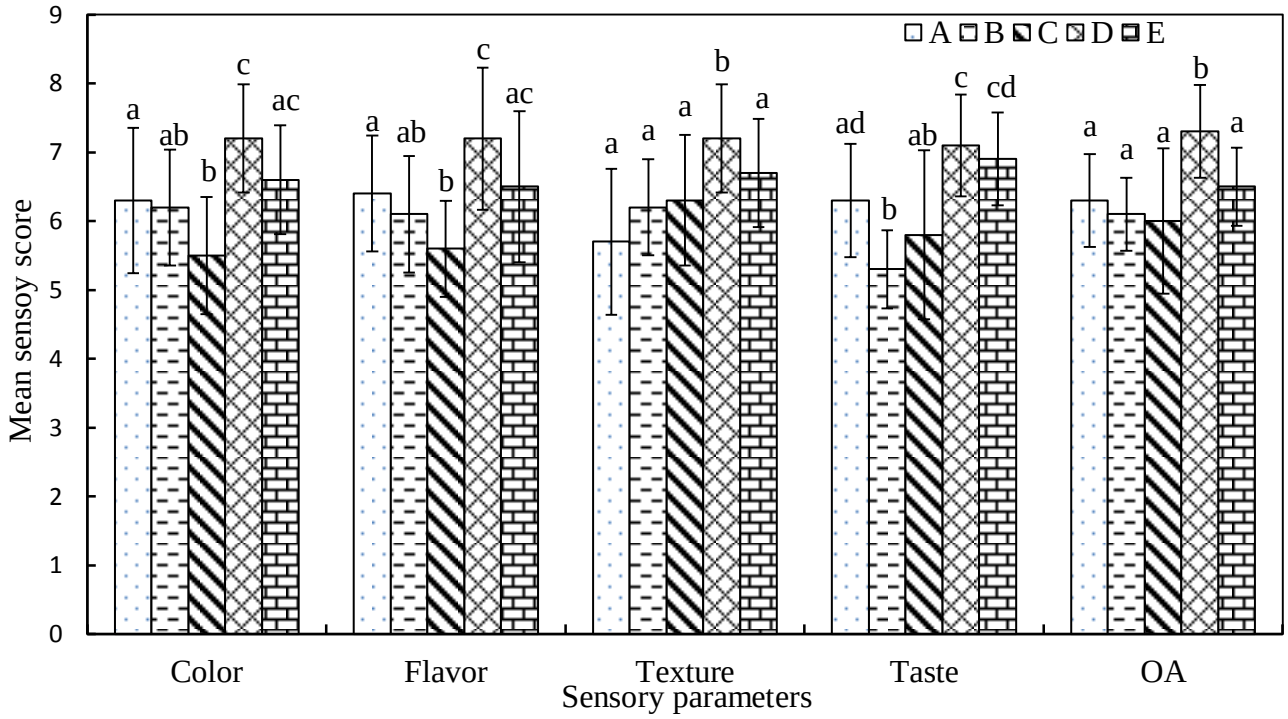

Fig. 4.2 Effect of proportion variation of oilseeds on sensory quality of *dhule achar*

[Note: A= Roasted 30% sesame +each 10% (perilla + flax + pumpkin seed + niger) powder + acid + spices mixture + salt, B= Roasted 30% perilla + each 10% (sesame + niger + flaxseed + pumpkin seed) powder + acid + spices mixture + salt , C= Roasted 30% flaxseed + each 10% (pumpkin seed + perilla + niger + sesame) powder + acid + spices mixture + salt, D= Roasted 30% pumpkin seed + each 10% (flaxseed+ niger + sesame + perilla) powder + acid +spices mixture + salt, E= Roasted 30% niger seed + each 10% (sesame + pumpkin seed + perilla + flaxseed) powder + acid + spices mixture + salt and OA represent overall acceptance

Similar alphabet above the bar indicates not significantly different ($p>0.05$). The error bars show the standard deviation.]

4.5.1 Color

The mean sensory score for color were 6.3±1.06, 6.2±0.79, 5.5±0.85, 7.2±0.79, 6.6±0.84 for samples A, B, C, D and E respectively. The obtained mean values are represented as bar diagram in Fig. 4.3. ANOVA of mean showed that there is significant difference ($p\leq0.05$) in color within the samples.

Sample D (7.2±0.79) had the highest score while the sample C (5.5±0.85) had the lowest. LSD showed no significant difference among samples A, B and E, D and E, B and

C. Sample D was significantly different from A, B and C except E. Similarly, the sample C also was significantly different from A, D and E. Sample D in which the pumpkin seeds powder was predominant was preferred by most panelists in term of color acceptance.

4.5.2 Flavor

The mean sensory score for flavor were 6.4±0.84, 6.1±1.1; 5.6±0.69, 7.2±1.03 and 6.5±0.85 for sample A, B, C, D and E respectively. The obtained mean values are represented as bar diagram in Fig. 4.3. ANOVA of mean showed that there is significant difference ($p \leq 0.05$) in flavor within the samples.

Sample D (7.2±1.03) had the highest score while the sample C (5.6±0.69) had the lowest. LSD showed that samples A, B and E; B and C; D and E were not significantly different ($p \leq 0.05$) among themselves. The sample D was significantly different from the samples A, B and C except E. Samples A and C, B and D and C and E were also significantly different among themselves. Thus, the sample which had high proportion of pumpkin seeds got the high score. It may be due to the unique flavor component present in pumpkin seeds.

Harper *et.al.* (2010), identified some sulfur compounds i.e. dimethylsulfide, methanethiol and methional which are considered to have the highest flavor impact in pumpkin seeds. Similarly, a sweet, nutty and typical roasted aroma may be due to pyrazines. Siegmund and Murkovic (2004) identified five pyrazines, 2-methylpyrazine, dimethylpyrazine, 2-ethylpyrazine, 2-ethyl-5(6)-methylpyrazine and 2-ethyl-3, 6-dimethylpyrazine, in the headspace of roasted pumpkin seeds, and theorized to significantly contribute to the overall aroma.

4.5.3 Texture

The mean sensory score for texture were 5.7±1.06, 6.2±0.79, 6.3±0.95, 7.2±0.79 and 6.4±0.69 for sample A, B, C, D and E respectively. The obtained mean values are represented as bar diagram in Fig. 4.3. ANOVA of mean showed that there is significant difference ($p \leq 0.05$) in texture within the samples.

Sample D (7.2±0.79) had the highest score while the sample C (5.6±0.69) had the lowest. The LSD showed that the sample D is significantly different from all samples i.e .A, B, C and E which were not significantly different among themselves. More preferences

to the sample D by panelists may be due to the smooth consistency and superior mouthfeel characteristics of the pumpkin seed.

4.5.4 Taste

The mean sensory score for taste were 6.3±0.82, 5.3±0.67, 5.8±1.23, 7.1±0.74 and 6.9±0.57 for samples A, B, C, D and E respectively. The obtained mean values are represented as bar diagram in Fig. 4.3. ANOVA of mean showed that there is significant difference ($p \leq 0.05$) in taste within the samples.

Sample D (7.1±0.74) had the highest score while sample B (5.3±0.67) had the lowest. LSD test showed that samples A and E; A and C; B and C; and D and E were not significantly different ($p \leq 0.05$) among themselves. Sample D was significantly different from all samples except E. Samples A and B; A and D; B and D; B and E; C and D; C and E were significantly different ($p \leq 0.05$) among themselves. More preference to the sample D by panelists may be due to more intense taste of pumpkin seed. Umano *et al.,* (2005), determined the formation of furan (2-methylfuran, 2-acetylfuran and 5-methylfurfural) and other compounds such as 2, 3-pentanedione, 2, 3-butanedione etc from heated glucose/cysteine and alanine increasing the characteristic taste. But sample B containing higher proportion of niger seeds scored least value may be due to its high fibre content.

4.5.5 Overall acceptance

The mean sensory score for OA were 6.3±0.67, 6.1±0.56, 6±1.05, 7.3±0.67 and 6.6±0.52 for samples A, B, C, D and E respectively. The obtained mean values are represented as bar diagram in Fig. 4.3. ANOVA of mean showed that there is significant difference ($p \leq 0.05$) in OA within the samples.

Sample D (7.3±0.67) had the highest score while the sample C (6±1.05) had the lowest. LSD test showed sample D was significantly different ($p \leq 0.05$) from all other samples. But there was no significant difference ($p > 0.05$) among samples A, B, C and E. At overall, the sample containing higher proportion of pumpkin seeds was preferred by most panelists and the sample C containing higher proportion of flaxseeds was least preferred by panelists.

The order of superiority in terms of color, flavor, texture, taste and overall acceptance of *dhule achar* from different oilseeds sources can be summarized as:

Color: Sample D> Sample E> Sample A> Sample B> Sample C

Smell; Sample D> sample A> Sample E> Sample B> Sample C

Texture: Sample D> Sample E> Sample C> Sample B> Sample A

Taste: Sample D> Sample E> Sample A> Sample C> Sample B

OA: Sample D> Sample E > Sample A> Sample B> Sample C

From sensory analysis based on mean score on five attributes, sample D containing pumpkin seeds as predominant oilseed was chosen as the best.

4.6 Optimisation of salt level

Salt level was optimized on the basis of sensory analysis. All ingredients were kept constant but salt level was varied (2%, 3%, 4%, 5% and 6%) in the recipe. The graphical representation of the sensory scores is given in Fig. 4.3. The statistical analysis shows that the overall characteristics (flavor, texture and taste) are significantly different ($p<0.05$) among the products due to variation in salt level. The sample containing 5% salt gave maximum sensory score in terms of the palatability and overall acceptance.

The sample containg 5% salt was significantly different ($p\leq0.05$) from the samples containing 2%, 3%, 4% and 6% salt. But the sample possessing 3%, 4% and 6% salt were not significantly different among themselves.

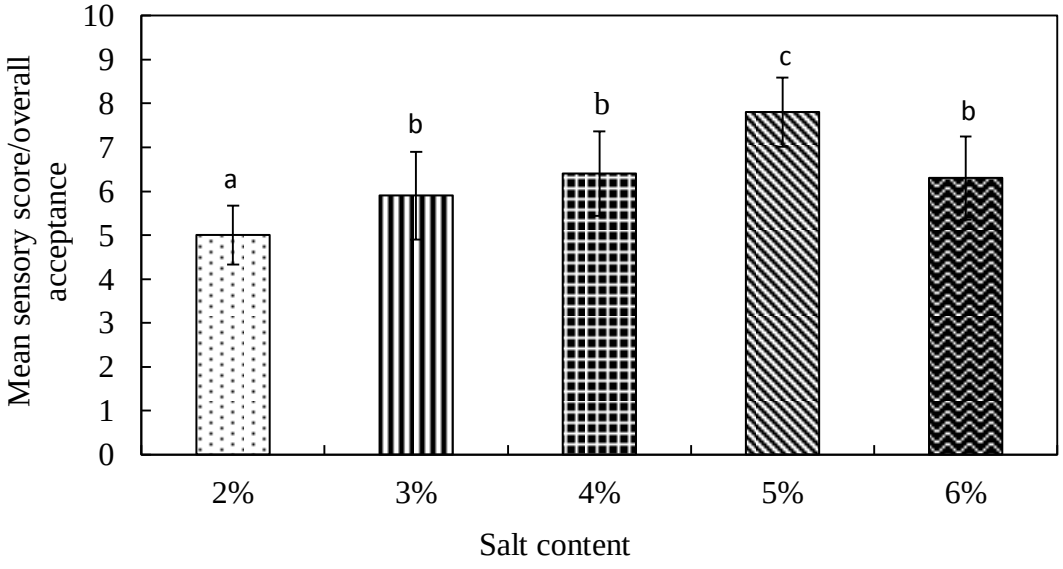

Fig. 4.3 Effect of salt on sensory quality/overall acceptance

4.7 Optimisation of acid (*Chuk amilo*)

Acid level in the product was optimized on the basis of sensory analysis. All ingredients were kept constant but acid level was varied in the recipe. The sensory scores of the *dhule achar* are shown in Fig. 4.4 and Appendix F.1.

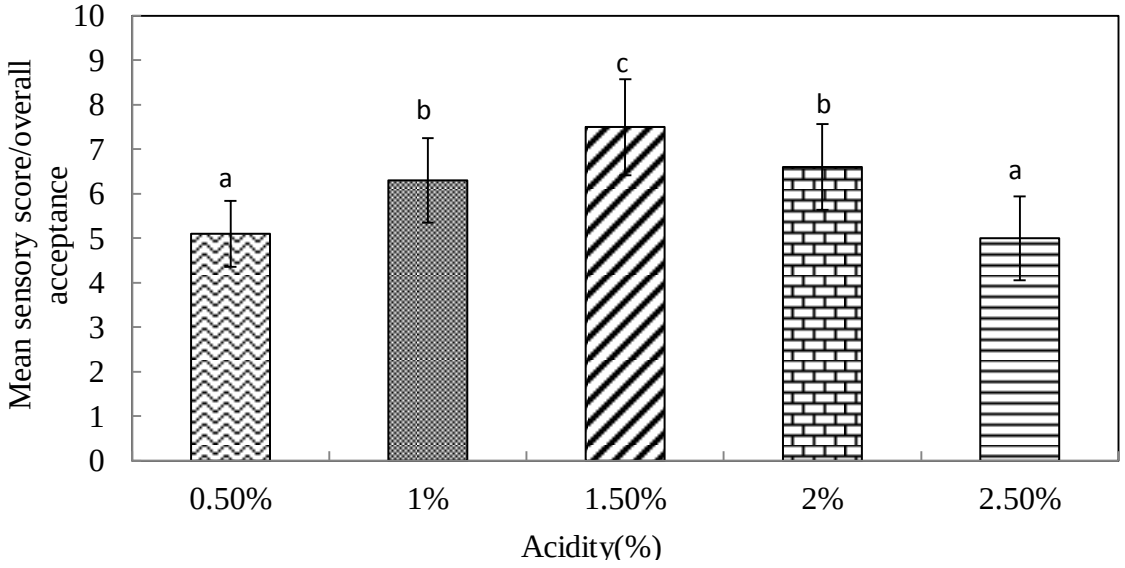

Fig. 4.4 Effect of acid on sensory quality/overall acceptance

Similar alphabet above the Fig. 4.3 and Fig. 4.4 indicates not significantly different ($p>0.05$).

The statistical analysis shows that the flavor, texture, taste and overall characteristics are significantly different ($p\leq0.05$) due to variation in acid level. The product containing 1.4% acidity gave maximum sensory score in terms of the palatability (Fig. 4.2). Sample containg 1.5% acidity was significantly different ($p\leq0.05$) from samples containing (0.5%, 1%, 2% and 2.5% acidity).

4.8 Chemical composition of *dhule achar*

Sample D (roasted 30% pumpkin seed + 10% flaxseed + 10% niger + 10% sesame + 10% perilla seed powder + acid+ spice mixture + salt) was chosen as the best formulation from sensory analysis and was analyzed for proximate and mineral composition which is presented in Table 4.2.

Moisture content of sample was found to be (12.26±0.28%). Slight high moisture content is due to the use of concentrated lemon juice (*Chuk amilo*). Protein content of *dhule achar* was found to be (24.3±0.19%). According to Swaminathan (2003), high protein diet is essential to fulfill the protein requirement of children and people.

Table 4.3 Proximate and mineral composition of sample D on wet basis* (per 100 g)

Parameters	Sample D
Moisture, %	12.26±0.28
Crude protein, % (N× 6.25)	24.3±0.19
Crude fat, %	30.80±0.24
Ash, %	7.5±0.13
Crude fiber, %	4.67±0.13
Total carbohydrate, %	20.47±0.98
Sodium, mg	2174±7.81
Potassium, mg	673± 2.34
Calcium, mg	242.56±6.02
Iron, mg	15.32±0.76

*Values are means of three determinations and figures with ± represent standard deviation.

Similarly, fat content of best formulation was found to be (30.80±0.24%). It means the product is rich in fat content. The oil of each ingredient is health beneficial. Linoleic acid which is abundant in sesame seed is one of the most important polyunsaturated fatty acids in human food because of its prevention of distinct heart vascular diseases (Boelhouwer, 1983). Sesamin present in the *achar* shows antioxidant (Yamashita *et al*., 2000), anticarcinogenic (Hirose *et al.,* 1992), blood pressure–lowering (Matsumura *et al.,* 1998), and serum lipid-lowering effects (Hirose *et al.,* 1991).

Pumpkin seed rich in plant sterols which have recently become of great interest due to the serum cholesterol-lowering effect (Miettinen *et al*., 1995; Jones *et al*., 2000). The seeds may also be beneficial against colon cancer (Awad *et al*., 1998). Flax seed also has recently gained good attention as a functional food due to the presence of omega 3- fatty acid. It is the richest dietary source of the lignan, secoisolariciresinol (SECO), a diphenolic compound when ingested by humans influences a wide range of biological systems that keep humans healthy (Muir, 2010).

Analysis shows high mineral content (7.5±0.13%) of *dhule achar* rich in sodium (2174±7.81 mg), potassium (673±2.34 mg), iron (15.32±0.76 mg) and calcium (242.56±6.02 mg). According to Swaminathan (2003), recommended dietary allowance of iron for children is 8 mg/day, for young girl is 15 mg/day and for pregnant and lactating women is 25 mg/day. Daily consumption of *dhule achar* may help to prevent severe

anaemic problem of children and women of Nepal. The sodium content of *dhule achar* is essentially contributed by the salt. Sodium is necessary for the transfer of molecules (e.g., amino acids) across membranes, for the transmission of nerve impulses, for the digestion of food and for muscular action (Heimbach, 1986).

4.9 Storage stability of *dhule achar*

The product was packed in two different packaging materials for its shelf life study and was stored at ambient condition (25±3˚C). For this purpose PP (80µm) and LDPE (65µm) were selected. After packing the product in PP pack, it was sealed but the product in LDPE was not sealed. It was just tied by the thread and kept as done in household level. At interval of 15 days acid value and peroxide value of each sample were determined. The effect of storage periods on acid value (AV) and peroxide value (PV) in both packaging material is presented Fig. 4.4 and 4.5.

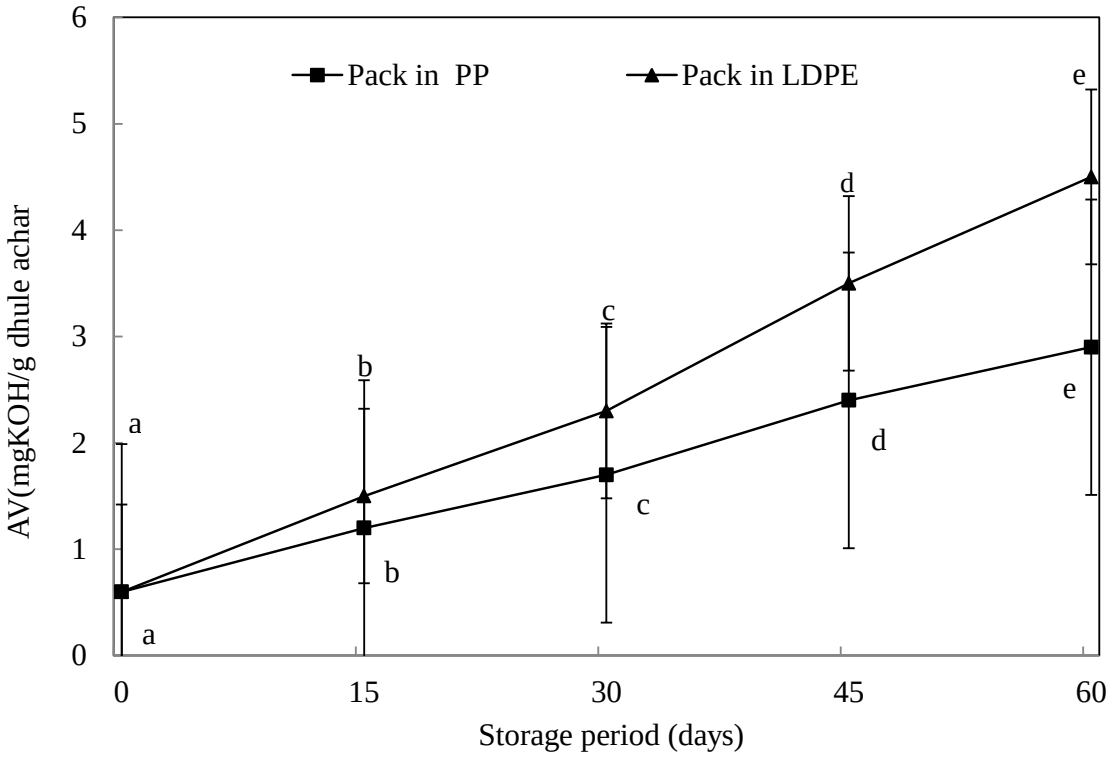

Fig. 4.5 Effect of storage period on acid value (AV) in PP and LDPE pack

Superscript for LDPE and subscript for PP along the row in Fig. 4.5 show significant difference among each 15 days at 5% level of significance.

As the storage period increased, acid value and peroxide value were increased significantly. Due to lack of time, shelf life evaluation was carried out only for 2 months. At the final day of analysis i.e. 60 days, the AV reached 2.9 mg KOH/g *dhule achar* in PP pack and 4.5 mg KOH/g *dhule achar* in LDPE pack. Acid value is an index to measure the free fatty acid. The FFA themselves are not toxic; however, the presence of FFA affects food quality. Consequently, measuring both indices is indispensible to control food quality and safty (Lundberg, 1961).

Similarly, PV reached 8.1 meq/kg *dhule achar* in PP pack and 13 meq/kg *dhule achar* in normal LDPE pack. In sealed PP pack, PV value is less than the rancid fat (10 meq/kg fat) but in normal LDPE pack the PV value seems higher (13 meq/kg *dhule achar*) indicating not fit for consumption. It can be said that by packing *dhule achar* in PP pack and sealing it, the shelf life of the product can be extended for few months.

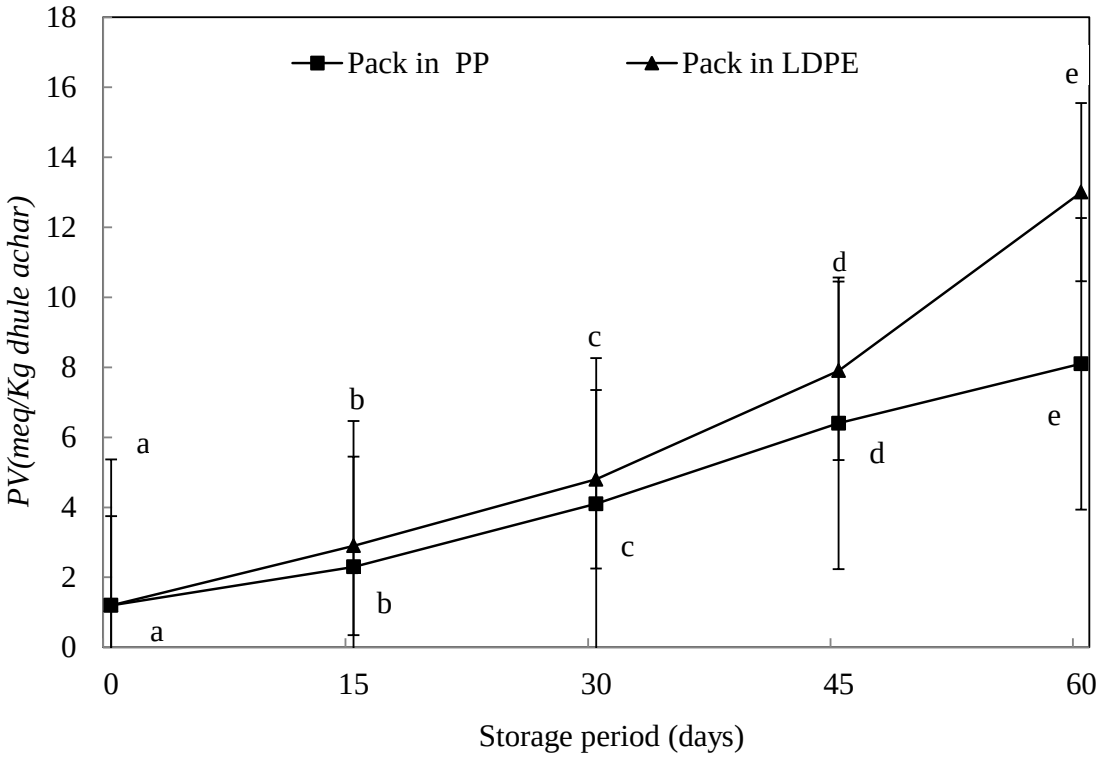

Fig. 4.6 Effect of storage period on peroxide value (PV) in PP and LDPE pack

Superscript for LDPE and subscript for PP along the row in Fig. 4.6 show significant difference among each 15 days at 5% level of significance.

Part V

Conclusions and recommendations

5.1 Conclusions

The following conclusions were drawn from the work:

1. Traditional pickle *dhule achar* can be prepared by utilizing the locally available ingredients like perilla, niger, sesame, flaxseed, pumpkin seed, ginger, turmeric, cumin, coriander, acid *(chuk amilo)* and salt.
2. Statistical analysis showed that there is significant effect of varieties of oilseeds on the sensory attributes of *dhule achar* like color, flavor, taste, texture and overall acceptance.
3. From the sensory analysis product C using equal proportion of oilseeds was found to be superior to others in first stage sensory analysis and sample D possessing higher proportion of pumpkin seeds was chosen as the best product in second stage product formulation.
4. Chemical analysis shows that *dhule achar* is rich in lipid, protein, iron, calcium, sodium and potassium.
5. Shelf life of the *dhule achar* can be extended for few months by packing and sealing it in polypropylene (80 μm) pack at ambient temperature (25±3˚C).

5.2 Recommendations

The following recommendations are made for further study:

1. Study on the antioxidant property of *dhule achar*.
2. Study on the nutritional quality (Digestibility, NPU, PER) of *dhule achar.*
3. Shelf life study of *dhule achar* using different packaging material.
4. Study on the improvement of sensory quality of *dhule achar*
5. Study on the effect of other acids such as citric acid, acetic acid (vinegar), juice of other citrus fruits etc. on the quality of *dhule achar*.

Part VI

Summary

Nepal is rich in traditional food and food habits. But many of them have no recorded manuals. Moreover many traditional foods are facing problems due to introduction of western foods. In many cases traditional foods may lack good image or have a poor perceived quality than the newer foods. A few of these foods have no doubt evolved into semi commercial commodity but most of them are still in a primitive stage. The changing life style and modernization is replacing the traditional foods with commercially promoted product. If this trend is continue, many of our traditional foods will soon be lost forever.

Dhule achar is a traditional pickle and popular in all community of Nepal. Its origination date backs to time immemorial and still not cleared. It is powdery product and prepared by blending roasted oilseeds with spices, salt and acid. It is generally consumed alongwith rice and breakfast items. Most preferably it is used in different cultural programmes such as *bratabandha, puja, sarad, kajkriya, marriage ceremony* etc. It is a good appetizer and supposed to maintain healty life as it contain high antioxidant property. Study was carried out to formulate, prepare and evaluate the quality evaluation and storage stability of the traditional pickle *dhule achar*. Survey was conducted among 114 persons specially womens of different parts of Nepal (Terhathum, Dhankuta, Sankhuwasava, Sunsari, Morang, Jhapa, Kathmandu, Makawanpur, Palpa, Chitawan etc.) about the preparation method of *dhule achar*.

Recipe and preparation method of *dhule achar* was then abstracted from the survey. From survey, it was known that there is no specific preparation method of *dhule achar* among all community. The oilseeds selected for this purpose also were varied. But the preparation method was not significantly varied. Oilseeds were cleaned, roasted, ground and sieved. Spices (cumin, coriander, turmeric, ginger and chilli) were also cleaned, sun dried, roasted, ground and sieved individually. The individual oilseed powder (70%) were mixed with spices (19%), salt (5%) and acid (*chuk amilo*) 6 g/100 g. Five samples of *dhule achar* were prepared from respective oilseeds and a sample was prepared by equal proportion of all oilseeds powder and it was also uniformly mixed with spices, salt and acid. Thus, six formulation of *dhule achar* were prepared and subjected to the sensory evaluation. Sensory evaluation (color, flavor, taste, texture and overall acceptance) was

done with 20 panelists including teachers and students of Central Campus of Technology on 9 point hedonic rating scale.

Statistical analyses showed there was significant effect of varieties of oilseeds (Perilla, flax, niger, sesame and pumpkin seeds) on the sensory attributes (color, flavor, taste, texture and overall acceptance). Sample C which was made by equal proportion of all oilseeds was most prefered by the panelists. Then five samples of *dhule achar* were prepared by taking one oilseed as predominant (30%) and keeping other oilseeds. Product D containing higher proportion of pumpkin seeds was chosen as the best product from the sensory analysis.

The best product was analysed for the proximate composition, mineral content and storage stability. Chemical analysis showed *dhule achar* has moisture (12.26±0.28%), crude protein (24.3±0.19%), crude fat (30.80±0.24%), ash (7.5±0.13%), crude fiber (4.67±0.13%) and total carbohydrate (20.47±0.98%). Similarly, mineral analysis shows calcium (242.56±6.02 mg), iron (15.32±0.76 mg), sodium (2174±7.81 mg) and potassium (673± 2.34 mg). The product was packed in PP pack of 80 µm and normal polythene (LDPE 65µm) pack for its shelf life study. PP pack was sealed while LDPE was only tied by thread and was kept at room temperature (25±3˚C). Frequent analysis of acid value and peroxide value of the product showed that the product in PP pack resulted in low acid value and peroxide value (2.9 mg KOH/g product and 8.1 meq/kg product) than packed in normal LDPE pack (4.5 mg KOH/g product and 13 meq/kg product) respectively at the final day of 60 day's storage. The cost evaluation of the product 100 g of *dhule achar* costs NRs. 29.56. Hence, highly nutritious traditional pickle *dhule achar* could be prepared at appropriate cost in household level.

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Appendices

Appendix A

Questions for the survey on *dhule achar*

Name of person: Age:

Name of place: Caste:

1) What do you know about *dhule achar*?
2) How long had you been preparing it?
3) What do you know about its origination?
4) What are the ingredients used?
5) Which ratio you mix these ingredients?
6) How do you prepare it?
7) For what purpose it is consumed?
8) How is it consumed?
9) Which acid do you use for it?
10) How long time does it remain and how it is packed and stored?

Appendix B

SENSORY EVALUATION CARD

Name of the Judge : …….

Date : ……..

Name of the Product : *Dhule achar*

Please test the given sample and score how much you prefer each one. Give points for your degree of preference for each parameter as shown below.

Sample	Color	Flavor	Texture	Taste	OA
A					
B					
C					
D					
E					
F					

Description of scale

9 = like extremely
8 = like very much
7= like moderately
6= like slightly
5= neither like nor dislike
4= dislike slightly
3= dislike moderately
2= dislike very much
1= dislike extremly

Comment if any

……

……

…….

Appendix C

Two factor ANOVA table for different sensory attributes (First stage)

1. Color

Table C.1 Two way ANOVA (no blocking) for color

Source of variation	d.f	s.s	m.s	Vr.	Fpr.
Panelist	9	11.000	1.222	1.22	0.306
Sample	5	28.333	5.667	5.67	<.001
Residual	45	45.000	1.000		
Total	59	84.333			

Since, Fpr<0.05, there is significant difference between the samples. So, LSD testing is necessary.

Table C.2 LSD table for color

Sample code	Mean score	Mean difference		LSD @0.05= 0.901
A	5.6	A-B> LSD*	B-D< LSD	D-E< LSD
B	6.3	A-C> LSD*	B-E< LSD	D-F< LSD
C	7	A-D< LSD	B-F< LSD	E-F< LSD
D	5.9	A-E< LSD	C-D> LSD*	
E	5.5	A-F< LSD	C-E> LSD*	
F	5.9	B-C< LSD	C-F> LSD*	

*> LSD signifies significant difference between the samples.

2. Flavor

Table C.3 Two way ANOVA (no blocking) for flavor

Source of variation	d.f	s.s	m.s	Vr.	Fpr.
Panelist	9	12.2667	1.3630	2.11	0.049
Sample	5	13.5333	2.7067	4.18	0.003
Residual	45	29.1333	0.6474		
Total	59	54.9333			

Since, Fpr<0.05, there is significant difference between the samples. So, LSD testing is necessary.

Table C.4 LSD table for flavor

Sample code	Mean score	Mean difference		LSD @0.05= 0.725
A	5.6	A-B< LSD	B-D< LSD	D-E< LSD
B	5.8	A-C> LSD*	B-E< LSD	D-F< LSD
C	6.8	A-D< LSD	B-F< LSD	E-F< LSD
D	5.4	A-E< LSD	C-D> LSD*	
E	5.5	A-F< LSD	C-E> LSD*	
F	6.1	B-C> LSD*	C-F< LSD	

*> LSD signifies significant difference between the samples.

3. Taste

Table C.5 Two way ANOVA (no blocking) for taste

Source of variation	d.f	s.s	m.s	Vr.	Fpr.
Panelist	9	17.8167	1.9796	2.42	.0025
Sample	5	32.2833	6.4567	7.88	<.001
Residual	45	36.8833	0.8196		
Total	59	86.9833			

Since, Fpr< 0.05, samples are significantly different. So, LSD is necessary.

Table C.6 LSD table for taste

Sample code	Mean score	Mean difference		LSD @0.05= 0.815
A	5.3	A-B> LSD*	B-D> LSD*	D-E< LSD
B	6.9	A-C> LSD*	B-E> LSD*	D-F< LSD
C	7.4	A-D< LSD	B-F> LSD*	E-F< LSD
D	5.9	A-E< LSD	C-D>LSD*	
E	5.6	A-F< LSD	C-E> LSD*	
F	6	B-C< LSD	C-F> LSD*	

*> LSD signifies significant difference between the samples.

4 Texture

Table C.7 Two way ANOVA (no blocking) for texture

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Panelist	9	18.2667	2.0296	3.44	0.003
Sample	5	8.8000	1.7600	2.98	0.021
Residual	45	26.5333	0.5896		
Total	59	53.6000			

Since, Fpr<0.05, samples are significantly different. So, LSD is necessary.

Table C.8 LSD table for texture

Sample code	Mean score	Mean difference		LSD @0.05= 0.614
A	5.9	A-B < LSD	B-D> LSD*	D-E> LSD*
B	6.1	A-C< LSD	B-E< LSD	D-F> LSD*
C	6.3	A-D> LSD*	B-F< LSD	E-F< LSD
D	7	A-E< LSD	C-D> LSD*	
E	5.9	A-F< LSD	C-E< LSD	
F	6	B-C< LSD	C-F< LSD	

*> LSD signifies significant difference between the samples.

5 Overall acceptance

Table C.9 Two way ANOVA (no blocking) for overall acceptancce

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Panelist	9	8.4833	0.9426	3.26	.0004
Sample	5	15.4833	3.0967	10.71	<.001
Residual	45	13.0167	0.2893		
Total	59	36.9833			

Since, Fpr<0.05, samples are significantly different. So, LSD is necessary.

Table C.10 LSD table for overall acceptance

Sample code	Mean score	Mean difference		LSD @0.05= 0.4844
A	5.6	A-B< LSD	B-D< LSD	D-E< LSD
B	6.3	A-C> LSD*	B-E< LSD	D-F< LSD
C	7	A-D< LSD	B-F< LSD	E-F< LSD
D	5.9	A-E< LSD	C-D> LSD*	
E	5.5	A-F< LSD	C-E> LSD*	
F	5.8	B-C< LSD	C-F> LSD*	

*> LSD signifies significant difference between the samples.

Appendix D

Two factor ANOVA table for different sensory attributes (Second stage)

1. Color

Table D.1 Two way ANOVA (no blocking) for color

Source of variation	d.f	s.s	m.s	Vr.	Fpr.
Panelist	9	11.9200	1.3244	2.14	0.051
Sample	4	15.3200	3.8300	6.19	<.001
Residual	36	22.2800	0.6189		
Total	49	49.5200			

Since, Fpr<0.05, there is significant difference between the samples. So, LSD testing is necessary.

Table D.2 LSD table for color

Sample code	Mean score	Mean difference	LSD @0.05= 0.714
A	6.3	A-B< LSD	B-D> LSD*
B	6.2	A-C> LSD*	B-E< LSD
C	5.5	A-D> LSD*	C-D> LSD*
D	7.2	A-E< LSD	C-E> LSD*
E	6.6	B-C < LSD	D-E< LSD

*> LSD signifies significant difference between the samples.

2. Flavor

Table D.3 Two way ANOVA (no blocking) for flavor

Source of variation	d.f	s.s	m.s	Vr.	Fpr.
Panelist	9	11.1200	1.2356	1.67	0.133
Sample	4	13.7200	3.4300	4.63	0.004
Residual	36	26.6800	0.7411		
Total	49	51.5200			

Since, Fpr<0.05, there is significant difference between the samples. So, LSD testing is necessary.

Table D.4 LSD table for flavor

Sample code	Mean score	Mean difference	LSD @0.05= 0.781
A	6.4	A-B < LSD	B-D> LSD*
B	6.1	A-C> LSD*	B-E< LSD
C	5.6	A-D> LSD*	C-D> LSD*
D	7.2	A-E< LSD	C-E> LSD*
E	6.5	B-C< LSD	D-E< LSD

*> LSD signifies significant difference between the samples.

3. Taste

Table D.5 Two way ANOVA (no blocking) for taste

Source of variation	d.f	s.s	m.s	Vr.	Fpr.
Panelist	9	4.8800	0.5422	0.73	0.678
Sample	4	22.4800	5.6200	7.57	<.001
Residual	36	26.7200	0.7422		
Total	49	54.0800			

Since, Fpr<0.05, samples are significantly different. So, LSD is necessary.

Table D.6 LSD table for taste

Sample code	Mean score	Mean difference	LSD @0.05= 0.781
A	6.3	A-B> LSD*	B-D> LSD*
B	5.3	A-C< LSD	B-E> LSD*
C	5.8	A-D> LSD*	C-D> LSD*
D	7.1	A-E< LSD	C-E> LSD*
E	6.9	B-C< LSD	D-E< LSD

*> LSD signifies significant difference between the samples.

4 Texture

Table D.7 Two way ANOVA (no blocking) for texture

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Panelist	9	10.3200	1.1467	1.76	0.111
Sample	4	11.7200	2.9300	4.49	0.005
Residual	36	23.4800	0.6522		
Total	49	45.5200			

Since, Fpr<0.05, samples are significantly different. So, LSD is necessary.

Table D.8 LSD table for texture

Sample code	Mean score	Mean difference		LSD @0.05= 0.732
A	5.7	A-B < LSD	B-D> LSD*	
B	6.2	A-C< LSD	B-E< LSD	
C	6.3	A-D> LSD*	C-D> LSD*	
D	7.2	A-E< LSD	C-E< LSD	
E	6.4	B-C< LSD	D-E> LSD*	

*> LSD signifies significant difference between the samples.

5. Overall Acceptance

Table D.9 Two way ANOVA (no blocking) for overall acceptance

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Panelist	9	7.5200	0.8356	1.87	0.089
Sample	4	10.7200	2.6800	6.00	<.001
Residual	36	16.0800	0.4467		
Total	49	34.3200			

Since, Fpr<0.05, samples are significantly different. So, LSD is necessary.

Table D.10 LSD table for overall acceptance

Sample code	Mean score	Mean difference	LSD @0.05= 0.732
A	5.7	A-B < LSD	B-D> LSD*
B	6.2	A-C< LSD	B-E< LSD
C	6.3	A-D> LSD*	C-D> LSD*
D	7.2	A-E< LSD	C-E< LSD
E	6.4	B-C< LSD	D-E> LSD*

*> LSD signifies significant difference between the samples.

Appendix E

Table E.1 Average sensory score and LSD for salt optimization for palatability

	% Salt					
Parameter	2	3	4	5	6	LSD
Palatibility taste	5a	5.9b	6.4b	7.8c	6.3b	0.835

Values are the means of 10 semi trained panelists. Means having the same subscript in a row didn't differ significantly (P<0.05) by LSD.

Appendix F

Table F.1 Average sensory Score and LSD for acid level optimisation

	Acidity (%)					
Parameter	1	1.2	1.4	1.6	1.8	LSD
Palatibility taste	5.1a	6.3b	7.5c	6.6b	5a	0.847

Values are the means of 10 semi-trained panelists. Means having the same subscript in a row did not differ significantly (P>0.05) by LSD.

Appendix G

Table G.1 Cost evaluation of *dhule achar*

Particulars	Cost (NRs/kg)	Cost of *dhule achar* (NRs/ 100 g)
Pumpkin seeds	150	4.5
Perilla seeds	180	1.8
Niger seeds	100	1
Sesame seeds	200	2
Flax seeds	170	1.7
Dried ginger	500	2
Turmeric	140	0.84
Cumin	400	1.6
Coriander	120	0.48
Red chilli	400	1.6
Salt	17	0.085
Acid *(Chuk amilo)*	600	3.6
Total		21.205
Processing and labor cost (20% of ingredients)		4.241
Packaging cost		2
Profit (10%)		2.12
Total cost/100 g		29.56