Addis Ababa University
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NUTRITIONAL COMPOSITION, PHYSICOCHEMICAL AND FUNCTIONAL PROPERTIES OF SOME CAPSICUM VARIETIES GROWN INETHIOPIA

BY

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A thesis submitted to the School of Graduate studies of Addis Ababa University in partial fulfillment of the requirement for the Degree of Master of Science in Food Science and Nutrition.

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ABSTRACT

This study was conducted to generate base line information on nutritional composition, physicochemical and functional properties of three capsicum varieties which are grown in Ethiopia. In relation to proximate composition, Marako fana (Capsicum annum), Bako local (Capsicum annum) and Oda haro (Capsicum annum) contained 9.156%, 9.043% and 8.744% moisture, 11.809%, 8.728% and 9.210% crude protein, 27.264%, 25.966% and 28.566% crude fiber, 11.1625%, 9.518% and 9.147% oleoresin, 89.011 mg/100g, 84.011 mg/100g and 84.818 mg/100g vitamin C, 1.685 mg/100g, 1.670 mg/100g and 1.754 mg/100g potassium, 27.156 mg/100g, 38.205 mg/100g and 54.565 mg/100g calcium and 7.236 mg/100g, 6.876 mg/100g and 9.554 mg/100g iron on wet weight basis. In case of physicochemical properties, functional properties and antinutritional factors, Marako fana, Bako local and Oda haro contained 648331 ICU, 520687 ICU and 478085 ICU color value, 0.217 %, 0.174% and 0.161% capsacinoide, 0.034%, 0.022% and 0.021% paprika, 13.96 N/pod, 4.66 N/pod and 4.21 N/pod firmness and 8.667%, 9.667% and 10.333% gel formation capacity 0.142 mg/100g, 0.164 mg/100g and 0.148 mg/100g tannin and phytate was below detection. The analysis of variance and LSD test done on the above results at $P \leq 0.05$ revealed that protein, oleoresin, color value, paprika content, firmness and gel forming capacity of Marako fana were significantly higher than the other two varieties while its tannin content was less. Potassium, calcium and iron content of Oda haro were significantly higher than the rest two varieties. But all the three varieties had comparable moisture, fiber, vitamin C and capsacinoide. Hence, Marako fana is preferable for large scale production of oleoresin and paprika. It is good coloring agent and can be used in food formulation like sauces in a better maner. Oda haro is nutritionally preferable for its potassium, calcium and iron. All have comparable pungency.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
CSA	Central Statistics Agency
EEPA	Ethiopian Export Promotion Agency
ESEF	Ethiopian Spice Extraction Factory
ICU	International Color Unit
IS	Indian Standard
ISO	International Standard Organization
LSD	List Significant Difference
ORAC	Oxygen Radical Absorption Capacity
SHU	Scovile Heat Unit
S.N.N.P.R.S	Southern Nations and Nationalities People Regional State
SPSS	Statistical Product and Service Solution

CHAPTER 1

INTRODUCTION

1.1 Background

Capsicum (pepper) is an autogamous plant, native to tropical America, which belongs to the family of solanceae (Rodriguez *et al.*, 2008).Capsicums or red peppers are the berries of capsicum plant and they form an indispensable ingredient of the culinary through out the world (Sanatombi and Jitendra, 2006).

The fresh green fruits and dried whole fruits or their powder with varying degrees of pungency, aroma and flavor are used in different cuisines of the world. Besides being an important food crop, red pepper is also used in pharmaceutical industries (Sanatombi and Jitendra, 2006), cosmetics industries and for ornamental in garden (Wesis, 2002). Capsicums are important food additives in many parts of the world, valued for their sensory attributes of color, pungency and aroma (Contreras and Yahia, 2000). Pepper used as food colorant has traditionally been in the form of ground powder, although today oleoresins are widely used (Hornero *et al.*, 2000b).

Pepper is the world second important vegetable ranking after tomatoes and it is the most produced type of spice used for flavoring and coloring food while providing vitamins and minerals (Rehima, 2006). They are essentially crops of the tropics and grow better in Africa, south and Central America, parts of the U.S.A and southern Europe (Jyothirmayi *et al.*, 2008). Nowadays, it is almost impossible to imagine the dishes of Asia and pacific region with out chili peppers while the traditional African sorghum or maize porridge would be tasteless with out them (Wesis, 2002).

Production of pepper is also well known in Ethiopia (Roukens, 2005). The history of pepper in Ethiopia is perhaps the most ancient than the history of any other vegetable product (EEPA,

2003). Ethiopians have strong attachment to dark red pepper, which has high value principally for its high pungency. The fine powdered pungent product is an indispensable flavoring and coloring ingredient in the common traditional sauce "wot" whereas; the green pod is consumed as a vegetable with other food items. There is a general belief among Ethiopians that a person who frequently consumes hot pepper has resistance to various diseases. It is in the daily diet of most Ethiopians. The average daily consumption of hot pepper by Ethiopian adult is estimated 15 gram, which is higher than tomatoes and most other vegetables (Rehima, 2006).

In addition to having major role in Ethiopians daily dish it also plays an important role in the national economy. It is an important cash crop today; on average 79% of pepper production is for market in S.N.N.P.R.S. It is a crop of high value in both domestic and export markets. Since it is a commercial and industrial crop, it generates employment to urban and rural workers (Rehima, 2006).

Oleoresin paprika and capsicum are extracted from red pepper (capsicum) for export purpose. The deep red colored and large podded cultivars (sweet/hot) have a very high processing demand in the country. The main processed product, oleoresin, is exported to different countries and the spiced ground is supplied to local market (EEPA, 2003).

1.2 Statement of the Problem

Ethiopia is the land of diverse climate and soil type that enables growth of several indigenous and exogenous spices, herbs, medicinal plants and other essential oil bearing plants. Despite the availability of the diverse agro ecologies of the country to produce these huge plant species and as they were playing a significant role on the national economy through generating considerable export earnings or import substitution, the research conducted on them is very limited due to various reasons (Girma *et al.*, 1998).

On the other hand an estimated of five million people of Ethiopia suffers form lack of vitamins (vitamin A and C) and essential minerals (iron and zinc). Vegetables are essential sources of most micronutrient and the only practical way to ensure their supply. They are valuable sources of minerals, vitamins, and proteins to the people like Ethiopia where the people experience malnutrition due to heavy dependence on cereals such as "tef", maize, wheat and other cereals. Vegetables like hot peppers and onion are sources of vitamins like vitamin A and C and minerals like potassium, sodium and calcium (Fekadu and Dandena, 2006).

In addition to the above Ethiopia is exporting oleoresin paprika and oleoresin capsicum to different countries by extracting from the capsicum variety Marako fana but the yield of this variety has been decreasing from time to time (Roukens, 2005).

This study therefore, was undertaken to generate base line information on the nutritional composition, physicochemical and functional properties of the capsicum varieties Marako fana, Bako local and Oda haro which are grown in Ethiopia.

1.3. Objectives

The general objective of this study was to generate information on the nutritional composition, physicochemical and functional properties of some capsicum varieties (Marako fana, Bako local and Oda haro) which are grown in Ethiopia.

The specific objectives of this study were to study:

- Physicochemical properties
- Nutritional and antinutritional composition
- Functional properties of the capsicum varieties (Marako fana, Bako local and Oda haro) grown in Ethiopia.

CHAPTER 2

LITERATURE REVIEW

2.1 Overall Spices Production

Spices are aromatic or fragrant products from tropical plants used to flavor foods or beverages. The main families of spices based on their importance on international bases are *cruciforae*, *lacuracea*, *leguminosae*, *myristicacea*, *mytacea*, *orchidacea*, *piperacea*, and *solanacea*. The *solanacea* contains about ninety genera and two thousand species of mainly tropical herbs, shrubs and small trees (Wesis, 2002).

There are around 40 to 50 spices of global economic and culinary importance. There are also many other species that are used in traditional cooking in the region of their natural occurrence but have yet to reach any significant trade. The major spice production is in the tropics from developing and least developed countries. There is also a very significant domestic consumption of spices in many spice-producing countries (FAO, 2009b).

The total volume of spices produced globally has been increasing gradually since 1998. There is global increment of 4.7, 4.8 and 4.9 million tons in 1998, 1999 and 2000 respectively. The biggest volume of spice comes from India. India produced 2.3, 2.2 and 2.1 million tons of spice in 2000, 1998 and 1999 respectively. China is the second biggest spice producer, showing an increase in productivity each year. Indonesia and Pakistan on the other hand were the third and fourth countries in spice production (FAO, 2009b). Asian countries in general dominate the world production of spices (Narong, 1995).

In Africa, Nigeria was the leading country in spice production in the years 1998, 1999 and 2000 with the production of 140,500, 141,500 and 146,500 tones respectively. Ethiopia was also the second with the production of 113,150, 114,150 and 110,960 tones respectively while Egypt was third (FAO, 2009b).

Table 2.1 Major countries in the world producing spice and their production in tones

Spices producing countries	Year		
	1998	1999	2000
India	2,163,600	2,243,700	2,255,800
China	523,828	556,288	584,871
Indonesia	258,167	251,802	260,902
Pakistan	179,451	160,755	208,328
Nigeria	140,500	141,500	146,500
Bangladesh	139,000	138,000	144,000
Ethiopia	113,150	114,150	110,960

(Source: FAO, 2009b)

Ethiopia has a wide range of agro ecological zone that enables the production of a wide variety of indigenous and exogenous spices.

Table 2.2 Production of various spices in Ethiopia

Spice type	Region			Total production (quintal)
	SNNPR	Oromiya	Amhara	
Ginger	374,210	3,154	0	377,364
Pepper	85718	66,736	148,524	300,978
Cummins	141	18,125	40,606	58,872
Korerima	55,927	326	0	56,253
Fenugreek	597	8,805	32,610	42,012
Turmeric	39,460	160	610	39,620

(Source: Roukens, 2005)

2.1.1 Capsicum Production and Utilization in the World, Africa and Ethiopia

When Columbus first landed on Hispaniola in 1942, capsicum was grown and used in virtually the whole of the Caribbean, Mexico, Central and Northern South America. Capsicum was taken to Europe by Columbus and then shortly afterwards to India and south East Asia on Portuguese trading voyages. Subsequently spread thought the tropics and warmer regions of the world. (Wesis, 2002).

Figure 2.1 Chile peppers by country of origin and association (Source: Netha and Reddy, 2000).

Nowadays, over 48% of the world capsicum (pepper) is produced in Asia (Rehima, 2006). India is the leading country in the world in pepper production with an area of 908 400 ha and production of 970800 tones of dry pepper (Sadhineni and Patil, 2007). India contributes about one forth of world's productions of pepper (Mahalingappa *et al.*, 2007). China and Indonesia are ranked second and third. India is also the major exporter of dry peppers, followed by china, and the major importing countries are the U.S.A and Germany (Rehima, 2006). In

Europe Hungary and Romania holds the first and second place while in Latin America Mexico became the leading country in pepper production (FAO, 2009b).

In Africa, North Africa, Senegal, Nigeria, Ghana and Kenya are the main producers of pepper. Ethiopia also cultivates pepper but her share in the world is insignificant compared to India that produced 1.5 million tones from 891, 800 hectares in 1992, Ethiopian's production in 2001/02 was only 779,624 tons harvested on 55,381 hectares. Red pepper is the leading vegetable and spice grown in the country. The central (eastern and southern Showa), western, north western (Wollege and Gojjam) and the southern part of the country are the potential pepper producing areas. Currently most of the products come from Alaba, Meskanina, Marko and Siltie zone which are found in southern part of Ethiopia. Birrshelko and Didessa valleys which are found in Wollege and Gojjam respectively also produce good amount of it. Chilli is well adapted in Gambella, Mizan Teferi and Tepi as a rain fed crop and in Gode as an irrigated crop (Rehima, 2006).Pepper production accounts for 34% of the total spice production in the three regions (Amhara, Oromiya and S.N.N.P.R.S) (Roukens, 2005).

Table 2.3 Cultivated area and production of pepper in Ethiopia

year	Area (ha)	Production (quintal)
2005/06	81544	1790283
2006/07	56914	101723
2007/08	75341	1223997

(Source: CSA, 2005, 2006, 2007, 2008)

Table 2.4 Yield of pepper in Ethiopia

year	Yield (quintal / hectare)
2005/06	22
2006/07	18
2007/08	18

(Source: CSA, 2005, 2006, 2007, 2008)

In terms of its utilization also capsicums are among the most heavily consumed spices throughout the world (Perva *et al.*, 2004). They are the most popular salad vegetables (Mantur *et al.*, 2007). They are widely accepted for use as a spice and staples in the diet (Van and Roozen, 1994). They are valued as a part of diet for different reasons in many parts of the world (Kirschbaum *et al.*, 2002). They add color, pungency and aroma to the cuisines of most countries in the world (Paik *et al.*, 2003). Titillating pungency and fascinating natural color of capsicum form an indispensable adjunct in every home all over the world (Sadhineni and Patil, 2007). They are the oldest and most important carotenoides (Deli *et al.*, 1996) which widely used as food colorants (Matsufuji *et al.*, 1998). They are also used as a spice and flavor ingredients in the food industries (Famurewa *et al.*, 2006). Dried ripe pods of many different verities of capsicum are utilized to prepare cayenne pepper, ground pepper and crushed red pepper (EEPA, 2003).

They are consumed as fresh fruits, canned as pickles, constitute condiments and sauces and dried as a powder in spice and seasonings (Kirschbaum *et al.*, 2002). Green chilli powders can be added to chips, finger fries, extruded products, sandwiches, pizzas, burgers etc. Ready

to eat and convenient products can be made with incorporation of green chili powders. Green chile powders can be used for making Asian and African diets (Jyothirmayi *et al.*, 2008).

It is now almost impossible to imagine the dishes of Asia and pacific region with out chili peppers while the traditional African sorghum porridge would be tasteless without them. It is the national spice of Ethiopia. An essential ingredient of the viscously hot "wot" and as one historian comment "with out chilli peppers one can not imagine a food, almost not an Ethiopian" (Wesis, 2002).

Berber is a popular Ethiopian seasoning prepared from red chili peppers, garlic, & other spices. It is sun-dried then mixed with more spices & used in wots. It is the source of the dark red color in all the hot dishes in Ethiopia. Finely minced onions, Berbere, water and vegetable oil are cooked long and slow over low heat until they become emulsified during the preparation of sauces of different kind. Although red pepper is the main ingredient in berbere, it is sun dried together with almost 20 other spices and herbs before being milled extra-fine. Awaze is a paste of Bereber served with meat dishes. Meten shiro is also a powder used for making sauces and prepared from the combination of Berebre and grinded pea with different proportion where the largest proportion is occupied by grinded pea. The green pod is also consumed as a vegetable with other food items (Anonymous, 2006).

2.2 Capsicum Anatomy, Growth Requirements and Cultivation Systems

Capsicums are the berries of the genus capsicum (family; solanacea) (Sanatombik and Sharma, 2008).This taxa includes both sweet cultivars eaten mainly as vegetables and hot cultivars often used as a spice (Durucasu and Tokusoglu, 2007). The most common species of capsicum are *capsicum annum*, *Capsicum frutescens*, *Capsicum chinense*, *Capsicum pubescens* and *Capsicum baccatum* (Netha and Reddy, 2000). The genus capsicum comprises greater than two hundred varieties (Contreras and Yahia, 2000).

In terms of its anatomy the pods are roughly triangular in shape in longitudinal cross-section with the base of the triangle at the point of attachment to the peduncle (stalk). The angles within this triangular shape may vary widely, the angle opposite the point of attachment of the peduncle being generally very acute, but becoming obtuse in rare cases, depending on the species (ISO, 1997).

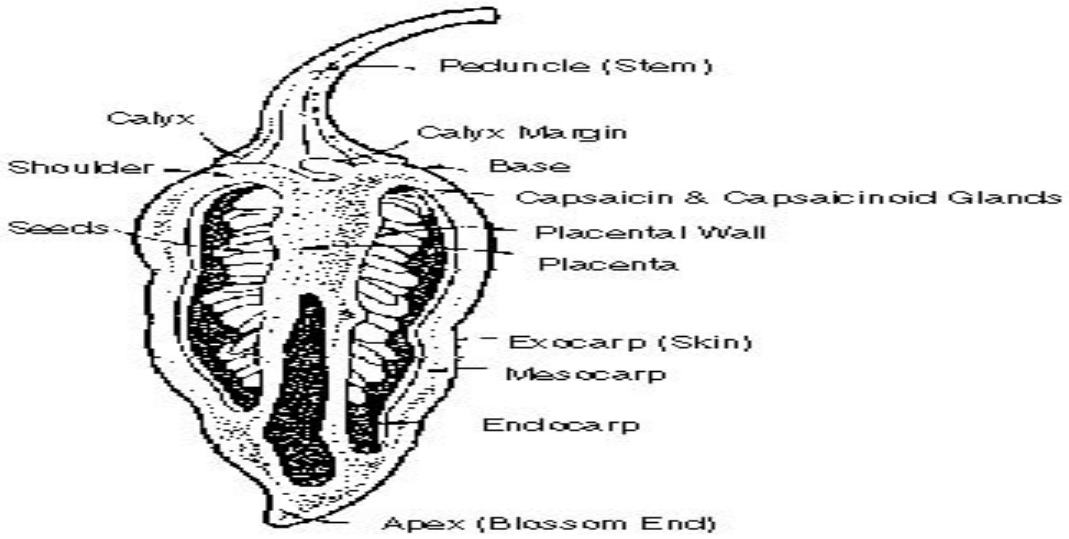


Figure 2.2 Anatomy of capsicum (Source: Anonymous, 2009)

The pods contain varying numbers of yellow/white, hard, disc-like seeds, 1 mm to 5 mm in diameter. The number and size of the seeds depend on the species. When mature, the seeds are attached individually to a relatively soft (spongy) central core within the pod by individual placenta (seed stalks), but in dried commercial chilies the seeds often become detached from the central core and move freely within the pods. The placenta is known to contain the highest concentration of the pungent capsaicinoids (ISO, 1997).

The mature pods may vary in color from dark blackish-red through orange-yellow-green, according to the species. The material pigmentation, particularly red, is affected by exposure to air and light during storage and the intensity decreases with time. Dimensions may vary

from 10 mm to 120 mm in length and 4 mm to 50 mm in diameter, depending on the species (ISO, 1997).

Pepper (capsicum) is an "annual" plant which grows at altitude ranging from 1400 to 2100 meter above sea level. Growing pepper requires soil hat is well drained and rich in organic matter, as well as 600-650 mm rainfall. It grows well on well prepared soil that is free form perennial weed (Roukens, 2005).

It is cold sensitive and hot dry weather is desirable for fruit ripening. Soil with a sandy loam or silt loam fixture is best (Francies, 2001). The seasons of high temperature and long photoperiod as commonly observed in tropical regions for a producing pepper enhance plant growth and seed yield. A warm night temperature (15-20^{0}c) is essential for normal flower development and formation of a well shaped fruits. The flowering, fruit set, fruit size and seed set are related to twenty four hour mean temperature as well as to day and night temperature fluctuations (Guohua and Kafkafi, 2003).

Materials of organic origin, for example top soil (remains of herbaceous, shrubs like and arboreal vegetation), chicken or hen bedding (mixture of chicken and or hen manure with materials used as bedding for hen houses) and worm humus showed satisfactory results as growth substrate. In addition to providing nutrients, the substrates to be used for seedling production must allow good water availability and water retention, and promote different gas exchange. In comparison to adult plants, seedlings have high demand for mineral nutrient, partly as the result of seedlings high growth rate (Grazia *et al.*, 2007).

Pepper is among the most susceptible horticultural plants to drought stress because of the wide ranging of transpiring leaf surface and high normal conductance having shallow root system. For high yields an adequate water supply and relatively moist soil are required during the entire growing season. A significant yield reduction was observed by limiting the amount of

water supply during the entire different growing periods. Low water availability prior to flowering of pepper reduces the number of flowers and retards the occurrence of maximum flowering. The water deficit during the period between flowering and fruit development reduces the final fruit production. When water source is scarce, pepper can be irrigated at lower water level, taking economic condition in to consideration (Gencoglan *et al.*, 2006).

Pepper grows in summer when evaporative demands are high and rain fall is scarce or non-exist. Hence along with nitrogen fertilizer, irrigation is the main factor conditioning crop growth, development and yield (Francies, 2001).

Production of pepper is well known in Ethiopia (Rehima, 2006). Pepper (capsicum) is propagated by raising seedlings in nursery. Seedlings are raised starting April and transplanted as the main rainy seasons begins, which is June/July. The seedlings are transplanted 40-50 days after planting. Planting is carried out at the beginning of the main rain season. In areas where rainfall is inadequate, supplementary irrigation is required. About three weeding seasons are recommended during the growth period. Depending on the area, harvesting starts four to five months from transplanting. The red pepper is harvested when it is fully red and starts to dry. After harvesting the pepper is dried. Shade drying is recommended for high quality oleoresin (Roukens, 2005).

2.3 Processing of Capsicum in Food Industries and at Home

Recently pepper is gaining greater importance in the global market because of its value added (processed) products and diverse uses (Sadhineni and Patil, 2007). Paprika is the powder of dry red pepper (Jaren *et al.*, 1999). Oleoresin is a liquid made by percolating a volatile solvent through a ground capsicum (paprika) and subsequent elimination of the solvent by vacuum evaporation. The solvent hexane is added in to the grinded powder of capsicum and allowed to extract the oleoresin for 4 hours. The hexane is then evaporated and the oleoresin is left at the

bottom of the container. Oleoresin consists of essential oils, resins and the components that provide pungency (ESEF, 2003a).

Crude oleoresin is separated into oleoresin paprika (sweet part) and oleoresin capsicum (hot or pungent part). Oleoresin paprika is a deep red viscous liquid with characteristic odor and color value of 100,000-120 ICU obtained from oleoresin by solvent extraction. Oleoresin capsicum is also a reddish viscous liquid with characteristic odor and color value not more than 4000 color units obtained from oleoresin by solvent extraction. When methanol is added to the oleoresin the hot part (oleoresin capsicum) will dissolve in it and the sweet part (oleoresin paprika) will settle at the bottom as a precipitate. The oleoresin paprika will be separated out by pouring the supernatant solution. The methanol in the supernatant solution is removed by evaporation and the oleoresin capsicum will settle at the bottom (ESEF, 2003a).

Not all cultivars can be used to produce oleoresin paprika. They must meet a series of appropriate agronomic and industrial requirements. The most valued of these is a high content in carotenoide as ultimately commercial value of paprika depends on its coloring capacity (Hornero *et al.*, 2000b).

Regarding pepper processing in Ethiopia, there were two extraction factories engaged in the production of paprika, capsicum, turmeric and ginger oleoresin: Ethiopia spice Extraction factory (ESEF) and KASSK Spices and Herbs extraction factory (KASSK). KASSK is a private factory which was built in 1991 and commenced production in 1997. The major extracted product for both factories is paprika oleoresin. The raw materials are supplied by local Merchants. The local merchants collect pepper mainly from Siltie zone, which is the major growing area for a Marko type of pepper. It contains oleoresin paprika (75%) and oleoresin capsicum (25%) (Roukens, 2005). The oleoresin is extracted from Marko using

hexane and segregated in to oleoresin paprika and oleoresin capsicum by using methanol (ESEF, 2003a).

KASSK mainly exported paprika oleoresin. The maximum export was 33.2 tons in 2002/03. ESEF also mainly export paprika oleoresin. In the last four years, from 2000/01 to 2003/2004 a total of 141 tons were exported by this factory. Paprika oleoresin is the principal export product in the country which accounted for a total of 196 tons and obtained USD 5,296.000. The major export destinations are Germany, Spain and Japan (Rehima, 2006).

Table 2.5 Ethiopian oleoresin paprika and oleoresin capsicum export

	ESEF		KASSK		Total	
Year	Oleoresin Paprika	Oleoresin Capsicum	Oleoresin Paprika	Oleoresin Capsicum	Oleoresin Paprika	Oleoresin Capsicum
	USD tons	USD tons	USD tons	USD tons	USD tons	USD tons
2001/01	498.8 17	0.0 0.0	111.5 3.5	0.0 0.0	609.3 20.5	0.0 0.0
2001/02	1494.0 40	9.1 1.7	0.0 0.0	0.0 0.0	1494.0 60.0	9.1 1.7
2002/03	877.4 34	10.6 2.2	982.0 32.2	2.1 0.6	1859.4 67.3	12.7 2.6
2003/04	891.8 30	11.6 2.2	441.6 18.6	0.0 0.0	1333.4 48.6	11.6 2.2
Total	3762 141	31 6	1535 55	2 1	5296 196	33 7

(Source: Roukens, 2005)

In Ethiopia pepper is also processed traditionally at home to prepare Berbere. The pepper is sun dried and mixed with ginger, cardamom, coriander, fenugreek seeds, cloves, cinnamon, garlic ,salt, cayenne pepper which are toasted at a low heat for a minute or so on a pan. This mixture will coarsely grinded and toasted at low heat for few minute. Finally, the mixture will be milled in to powder. This powder is commonly called Berbere. Awaze is a food item

prepared from Berbere. Berbere is mixed with water and some times alcoholic drinks are added as flavoring agent (Anonymous, 2006).

2.3.1 Health Benefits of Capsicum

Carotinoids of capsicum ingested in the diet have important nutritional and physiological functions through their general role as antioxidant and as provitamin A in certain cases (mainly in B-carotene). Thus they have been related with decreased risk of certain types of cancer, anti ulcer effect, activation of the immunological system (Hornero *et al.*, 2000a).

Capsicum lowered gastric ulcer by decreasing acid pepsin and increasing mucous secretion (Das and Deka, 2008). Capsaicin, as the most important among pungent principle, is a powerful irritant of the receptors participating in circulatory and respiratory reflexes and is used in stimulating medicines and food preparation. It gives relief from pain sensations when applied to scar tissue or other pain full conditions (Perva *et al.*, 2004).

2.4 Physicochemical, Nutritional, Antinutritional and Functional properties

2.4.1 Physicochemical Properties

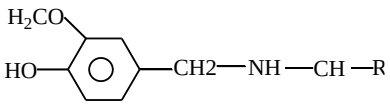
The genus capsicum has a variety of species which add color, pungency and aroma to the cuisine of most countries in the world (Paik *et al.*, 2003).

The pungency of pepper is due to a group of closely related alkaloids called capsaicinoides found only in the genus capsicum (Pordesimo *et al.*, 2004; Sanatombik and Sharma, 2008). There are more than ten closely related capsaicinoides but capsaicin (8-methyl-N-vaniyl-6-noneamide) and dihydrocapsaicin are responsible for 90% of the pungency. They are biosynthetically synthesized in the placenta of the fruit (Contreras and Yahia, 2000).

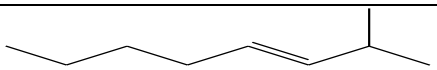
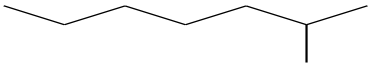
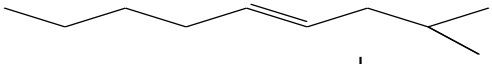
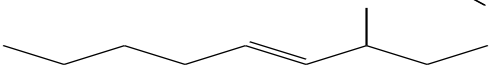
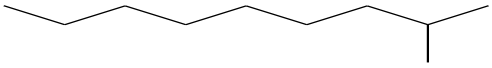
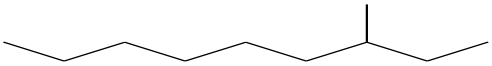
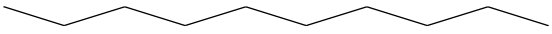
Capsaicinoides exist from 0.05% in the mildy pungent types to as high as 1.3% in the hottest (Perva *et al.*, 2004).The hotter the pepper the higher it's content of capsaicin. Cayenne pepper is the strongest red pepper variety of capsicum family with paprika being the mildest

(Anonymous, 1996). Capsaicinoides are produced only in the fruit and their function is tightly linked to direct defense of the developing embryo (Levey *et al.*, 2007). Capsaicinoide production increases with maturity until a maximum, and then decreases by rapid turn over and degradation of up to 60% (Contreras and Yahia, 2000).

Table 2.6 Chemical structures of different capsaicinoids and their analogues



General structure of capsaicinoids

R	Name of the capsaicinoide
	Capsaicin
	Dihydrocapsiaicin
	Nordihydrocapsiacin
	Homocapsiacin I
	Homocapsiacin II
	Homodihydrocapsaiacin I
	Homodihydrocapsiacin II
	N-Vanillyl octanamide
	N-Vanillyl nonanamide
	N-Vanillyl decanamide

(Source: Contreras and Yahia, 2000).

Capsaicin, the major pungent compound of hot pepper fruit, is an amide derivative of vanillylamine and 8-methylnontrans-6-enoic acid. The vanillyamine moiety of capsaicin is

biosynthetically derived from L-phenyl alanine while the branched fatty acid moiety is derived form valine (Bernal and Ros, 1996).Capsaicin is a potent chemical that can survive form both cooking and freezing but apart from burning generation (Famurewa *et al.*, 2006). Sun drying is found to degrade capsaicin pigment (Jyothirmayi *et al.*, 2008).

Capsaicin which is the main component of the fruits of capsicum plays an important role in the physiological and pharmacological effects on the sensory and cardiovascular system (Paik *et al.*, 2003). It is responsible for most of its beneficial effect on human physiology (Anonymous, 1996).

It triggers the brain to provide endorphins, natural painkiller that promotes a sense of well being (Famurewa *et al.*, 2006).

Table 2.7 Capsaicin, Dihydrocapsaicin and Capsiacinoide contents of dried Jalapeno pepper

Type of the pepper	Capsaicin (mg/g of dry fruit)	Dihydrocapsaicin (mg/g of dry fruit)	Capsiacinoide (mg/g of dry fruit)
Dried Jalapeno pepper	17.093	2.180	26.340

(Source: Contreras and Yahia, 2000)

Natural extract of red pepper with 40% capsaicinoide content can be disseminated in the atmosphere as a sensory irritant via a smoke composition. Being a natural extract and less toxic as compared to cyanide agent (tear gas), it can be used in riot control and hostage rescue situation (Kulkarni *et al.*, 2006).

In general, peppers have a wide range of pungency which is affected by the environment in which they were grown (Famurewa *et al.*, 2006). The amount of pungency is expressed by scovile heat index (ISO, 1995). Wilbur Scoville-1912 developed a method to measure the heat level of chile peppers. The test is named after him, the “Scoville Organoleptic Test”. The number of Scoville heat units (SHU) indicates the amount of capsaicin present in it. Bell

peppers rank at 0 (SHU). Naga Jolokia**,** chilli pepper that grows in northeastern India and Bangladesh, is the hottest chili in the world, measuring over 1,000,000 SHU. Pure capsaicin, measures 16,000,000 SHU (Netha and Reddy, 2000).

Red pepper market value depends largely on the red color. Although does not necessarily reflect nutritional, flavor or functional properties, it is related to consumer preference based on the appearance of the product. Moreover color is a quality characteristic that reflect the combined effects of oil content, water content, raw material proportion (proportion of pod and seed) as well as the technological factors such as drying conditions, grinding pressure, dispersion of granules etc (Chen *et al.*, 1999).

The red pepper fruit has been used since ancient times as a source of pigments to add to or change the color of food staffs, making them more attractive and acceptable for the consumer. The fruits of capsicum owe their red color to carotenoid pigments that are synthesized massively during fruit ripening (Hornero *et al.*, 2000b).

All the carotenoide pigments present in pepper are C_{10} isoprenoids containing nine conjugated double bonds in the central chain, although with different end groups which change the chromophore properties of each pigment, allowing them to be classified in to two isochromic families; red and yellow. The red fraction contains pigments exclusive to the capsicum genus (capsanthin, capsanthin-5, 6- expoxide, and capsorubin) and the yellow fraction comprises the rest of the pigments (zeaxanthin, violaxanthin, antheraxanthin, β- crypotoxanthin, B-carotene and cuucurbitaxanthin A) which acts as precursors of the former pigment (Hornero *et al.*, 2000a; Hornero *et al.*, 2000b).

The red carotenoids, capsanthin, capsorubin and capsanthin-5, 6- expoxide accounts for 30-40% of the total carotenoide in fully ripe fruits (Matsufuji *et al.*, 1998).

A capsanthin is one of the major carotenoides of red paprika essterified with fatty acid in ripe fruits. The esterifed capsanthin increases as the fruit is ripened (Matsufuji *et al.*, 1998).In general, red pepper and traditionally the paprika obtained from certain verities and more recently the oleoresin are good source of carotenoid pigments for use in food stuffs (Hornero *et al.*, 2000a).

Texture, in particular crispiness of the pepper is also an important quality attribute to the consumers. The major post harvest problem with this crop is excessive softening that may cause shrinkage, drying and pathological disorders which severely reduce the acceptability of the product (Morais *et* al., 2008).

The number and size of seeds attached to the placenta and size of the pod in general are other physical characteristics that vary depending on species type (ISO, 1997).

2.4.2 Nutritional Composition

Chili peppers have high nutritional value in terms of Vitamin A and C and minerals like sodium, potassium and calcium (Askari *et al.*, 1995). The nutritional value of hot pepper needs special attention not only its pungency or color. It is a rich source of vitamin. Both the hot and sweet peppers contain more vitamin C (Rehima, 2006). Capsicum contains fats, proteins, vitamin A, ascorbic acid, B-complex vitamins, potassium and falvonoid (Anonymous, 1996). It also contains Iron, copper, Zinc, manganese, sodium, calcium and magnesium (Rubio *et al.*, 2002).

The utilizable carbohydrate (56.2%) and crude fiber (22.5%) contents of dried capsicum annum on wet weight basis are very large proximate components as compared to moisture (13.4%), crude protein (12.8%), total ash (5.7%) and crude fat (11.9%) contents (FAO, 2009a).

Table 2.8 Chemical composition of green chili powder

Parameter	Values
Moisture (%)	7.20 ± 0.31
Total ash (%)	5.44 ± 0.26
Acid insoluble ash (%)	0.016 ± 0.00
Acidity(%) as citric acid	0.23 ± 0.016
Crude fat (%)	7.52 ± 0.54
Crude protein (%) NX6.25	13.52 ± 0.23
Crude fiber (%)	36.19 ± 0.49
Carbohydrate (%)	30.27 ± 0.36

(Source: Jyothirmayi *et al.*, 2008)

Table 2.9 Range of contents of macro and micro elements present in capsicum (mg/100gm)

Elements	Range of contents
Macro elements	
Na(sodium)	0.5-5.00
K(potassium)	177-260
Ca(calcium)	9-12
Mg(Magnesium)	10-14
Microelements	
Fe	0.4-0.75
Zn	0.12-0.26
Cu	0.065-0.104
Mn	0.10-0.125

(Source: Rubio *et al.*, 2002)

Table 2.10 FAO Vegetable and vegetable products Food composition table (composition per 100 gram of edible portion)

Item No.	Food and Description	Food Energy (cal)	Moisture (g)	Protein (g)	Fat (g)	Carbohydrate (g)	Fiber (g)	Ash (g)	Calcium (mg)	Phosphorus (mg)	Iron (mg)	B-carotene Equivalent (mg)	Ascorbic acid
740.	Peppers (Pipers umbellatum), leaves	69	82.1	4.6	3.4	8.2	1.8	1.7	169	40			
			(1)	(1)	(1)		(1)	(1)	(1)	(1)			
741.	Peppers, hot (Capsicum pubescens), raw	42	87.4	1.1	.1	10.7	1.4	.7	5	18	1.2	330	40
			(2)	(2)	(2)		(2)	(2)	(2)	(2)	(2)	(2)	(2)
			--	--	--		--	--	--	--	--	--	--
	Peppers, red; tabasco												
	(Capsicum frutescens), fruit:												
742.	Raw	94	74.2	4.1	2.3	18.0	6.0	1.4	58	101	2.9	7, 140	121
			(14)	(13)	(13)		(13)	(13)	(13)	(11)	(11)	(10)	(18)
			61.5–77.6	3.3–5.2	.6–7.0		4.5–10.1	1.2–2.4	34–110	86–105	2.5–4.0	5,000–9, 280	70–250
743.	Dried	346	9.8	12.5	11.5	61.5	23.3	4.7	187	330	16.7	14, 250	12
			(17)	(15)	(15)		(12)	(14)	(13)	(11)	(11)	(9)	(15)

Item No.	Food and Description	Food Energy (cal)	Moisture (g)	Protein (g)	Fat (g)	Carbohydrate (g)	Fiber (g)	Ash (g)	Calcium (mg)	Phosphorus (mg)	Iron (mg)	B-carotene Equivalent (mg)	Ascorbic acid (mg)
	Peppers, sweet (Capsicum annuum), fruit:												
744.	Raw, immature, green.	48	86.1	2.0	.8	10.3	2.6	.8	29	61	2.6	180	140
			(42)	(41)	(41)		(34)	(41)	(37)	(30)	(26)	(6)	(30)
			67.4–96.0	.5–3.9	.1–4.3		.2–6.3	3–1.6	12–70	20–120	.7–2.8	170–200	113–316
745.	Dried	331	13.4	12.8	11.9	56.2	22.5	5.7	87	128	8.7	2, 840	93
			(6)	(6)	(5)		(3)	(6)	(4)	(2)	(2)	(2)	(3)
			10.1–15.8	11.0–15.8	9.4–15.6		22.4–22.8	3.4–7.2	65–106	--	--	--	52–177
746.	Peppers, sp. (Capsicum abyssinicum), fruit, raw.	100	72.8	3.0	2.4	20.2	9.2	1.6	19	110	5.6	1,410	147
			(2)	(2)	(2)		(1)	(2)	(2)	(1)	(2)	(1)	(2)
			72.7–72.9	2.6–3.3	1.7–3.1			--	14–24		4.6–6.5		--

(Source: FAO, 2009a)

2.4.3 Antioxidant Activity and Anti nutritional Factors of Pepper

Many studies have demonstrated that peppers contain a wide array of phytochemicals like. But many pepper species and cultivars have not been analyzed for these important compounds. The phytochemical changes that occur during maturation and the resultant effect of the antioxidant activity are important dietary considerations that may affect the consumption of different pepper types. Phytochemicals that are important antioxidant components of plant based diet other than traditional nutrients may reduce the risk of degenerative diseases (Howard *et al.*, 2000).

Fresh peppers are an excellent sources of vitamin A and C as well as neutral and acidic phenolic compounds which are important antioxidants for a variety of plant defense response. Levels of these compounds can vary by genotype, maturity and are influenced by growing conditions and losses after processing. The role of ascorbic acid in the diet is thought to be significant in preventing common degenerative conditions including cancer, heart diseases, cataracts and immune functioning change due to its antioxidant nature (Howard *et al.*, 2000). This vitamin is found in large amount in pepper (Famurewa *et al.*, 2006). Ascorbic acid known as vitamin C needs to be consumed via foods like pepper as it is not produced in the human organism and important as an antioxidant (Antoniali *et al.*, 2007).

Table 2.11 Total Phenolics and Flavenoides in Bulgarian pepper

Vegetable	Latin name	Total phenolics (mg/100g)	Flavenoide (mg/100g)
Red pepper	Capsicum annuum	173.2	13.7
Green pepper	Capsicum annuum	246.5	27.4

(Source: Marinova, 2005)

Table 2.12 Antioxidant activity of some vegetables

Vegetable type	Antioxidant activity based on Oxygen Radical Absorption Capacity (ORAC) µmol TE/g
Carrot	60
White cabbage	61
Tomato	67
White onion	85
Red pepper	97
Cauliflower	102
Beet	115
Broccoli	126
Purple onion	143
Spinach	152
Green pepper	154

(Source: Boxin *et al*, 2002)

The antioxidant activity of green pepper is grater than carrot, white cabbage, tomato, white onion, red pepper, cauliflower, beet, broccoli, purple onion and spinach based on oxygen radical absorption capcity (Boxin *et al*, 2002).

The bioavailability of nutrients is affected by anti nutritional factors such as phytic acid, tannin, oxalate, phenolic compounds and enzyme (trypsin, chymotrypsin, α -amylase) inhibitors (Ramakrishna et al., 2008).

Tannins were found to reduce weight gain and feed conversion (Idris *et al.*, 2005). Phytate has long been recognized as an antinutritional factor affecting the bioavailability of major minerals such as Ca and P and trace ones such as Zn, Fe, Cu and Mn (Eltayeb *et al.*, 2007).

It binds with trace and macro elements. It can also form complexes with proteins, proteases and amylases of the intestinal tract, thus inhibiting proteolysis. Phenolic compounds are responsible for the bitterness and astringency of many foods (Ramakrishna *et al.*, 2008)

An adequate mineral absorption is important especially for infants, children, elderly people and people in clinical situation (Idris *et al.*, 2007). Hence elimination or inactivation of such antinutritional compounds is absolutely necessary to improve the nutritional quality.

Soaking in water and storing under carbon dioxide atmosphere are found to reduce tannin content. On the other hand fermentation is also a traditional method which reduces phytic acid content (Eltayeb *et al.*, 2007).

2.4.4 Functional Properties

- **Water Absorption Capacity**

Water absorption capacity describes flour – water association ability under limited water supply (Oladele and Aina, 2007). It gives an indication of the amount of water available for gelatinization. Water binding capacity is a useful indication of whether flour or isolates can be incorporated into aqueous food formulations (Udensi and Okoronkwo, 2006).

- **Oil Absorption Capacity**

Oil absorption is an important property in food formulations because fats improve the flavor and mouth feel of foods (Odoemelam, 2005).

- **Viscosity**

Viscosity is a measure of the resistance to flow of a paste. It is an indicative of a paste thickness and consistency (Ikujenlola and Fashakin, 2005).

❖ **Foaming Capacity and Stability**

The formability of flours has been shown to be related to the amount of native protein. Native protein gives higher foam stability than the denatured protein. It is related to the amount of solubilized protein (Odoemelam, 2005).

❖ **Emulsion Capacity and Stability**

An oil-in-water emulsion is a two-phase system in which the hydrophobic phase (oil droplets) is surrounded by a continuous aqueous phase. It is assumed that, once formed, the droplets in the emulsion are stabilized by a protein film at the interface (Njintang *et al.*, 2007).

❖ **Bulk Density**

Bulk density is a measure of heaviness of a flour sample. It gives an indication of the relative volume of packaging material required. Generally, higher bulk density is desirable for the greater ease of dispersibility and reduction of paste thickness (Udensi and Okoronkwo, 2006).

❖ **Gel Formation**

The gelling capacity of flours is related to denaturation, aggregation and thermal degradation of starch (Odoemelam, 2005).

❖ **Dispersibility**

The dispersibility of a mixture in water indicates its reconstitutability. The higher the dispersibility, the better it would be (Kulkarn *et al.*, 1991).

CHAPTER 3

MATERIALS AND METHODS

3.1 Source of Research Materials Collection and Sample Preparation

Three capsicum varieties, Marako fana (Capsicum annum), Bako local (Capsicum annum) and Oda haro (Capsicum annum) which were important for the analysis were collected from Bako agricultural Research Center where the annual rain fall, relative humidity, mean daily temperature and soil type are 1231.1mm, 62.8, 22^0c and nitosol (brown reddish) respectively (Bako, 2007).

Figure 3.1 Marako fana

Figure 3.2 Bako local

Figure 3.3 Oda haro

There were thirty sacks for each variety and out of these sacks five sacks were selected based on random sampling system. The number of sacks (n) to be taken for sampling varies depending on the size of the lot (N) as shown in the table below (ISO, 1980).

Table 3.1 Number of container to be taken from a given lot size

Lot size(N)	Number of sacks to be taken(n)
1 to5 sacks	All sacks
6 to 49 sacks	5 sacks
50 to100 sacks	10% sacks
Over 100 sacks	The square root of sacks

(Source: ISO, 1980)

But there were thirty sacks and hence only five sacks were selected based on the above table. These five sacks were selected by taking every 6^{th} sack where the value 6 was obtained by dividing 30 by 5. From every 6^{th} sack also the samples were taken from the top, middle and bottom part of the sack. These samples taken from every 6^{th} sack were mixed together for each variety (ISO, 1980). The mixed samples were packed by polyethylene packaging material and transported to the Ethiopian Health and Nutrition Research Institute laboratory where the analysis was done.

The samples which were collected were grinded by using Iika-weke grinding mill of model M2059. The grinded powders of capsicum samples were sieved through 1mm sieve. These sieved powders were collected and packed in dry polyethylene bags (ISO, 1981). All analyses were done by using analytical grade chemicals and regents.

Figure 3.4 Grinded Marako fana

Figure 3.5 Grinded Bako local

Figure 3.6 Grinded Oda haro

3.2 Flow Chart of the Methodology

Proximate composition, physicochemical properties, functional properties and antinutritional factors of the grinded powders of Marako fana, Bako local and Oda haro were analyzed by following the flow chart as shown in the figure 3.7.

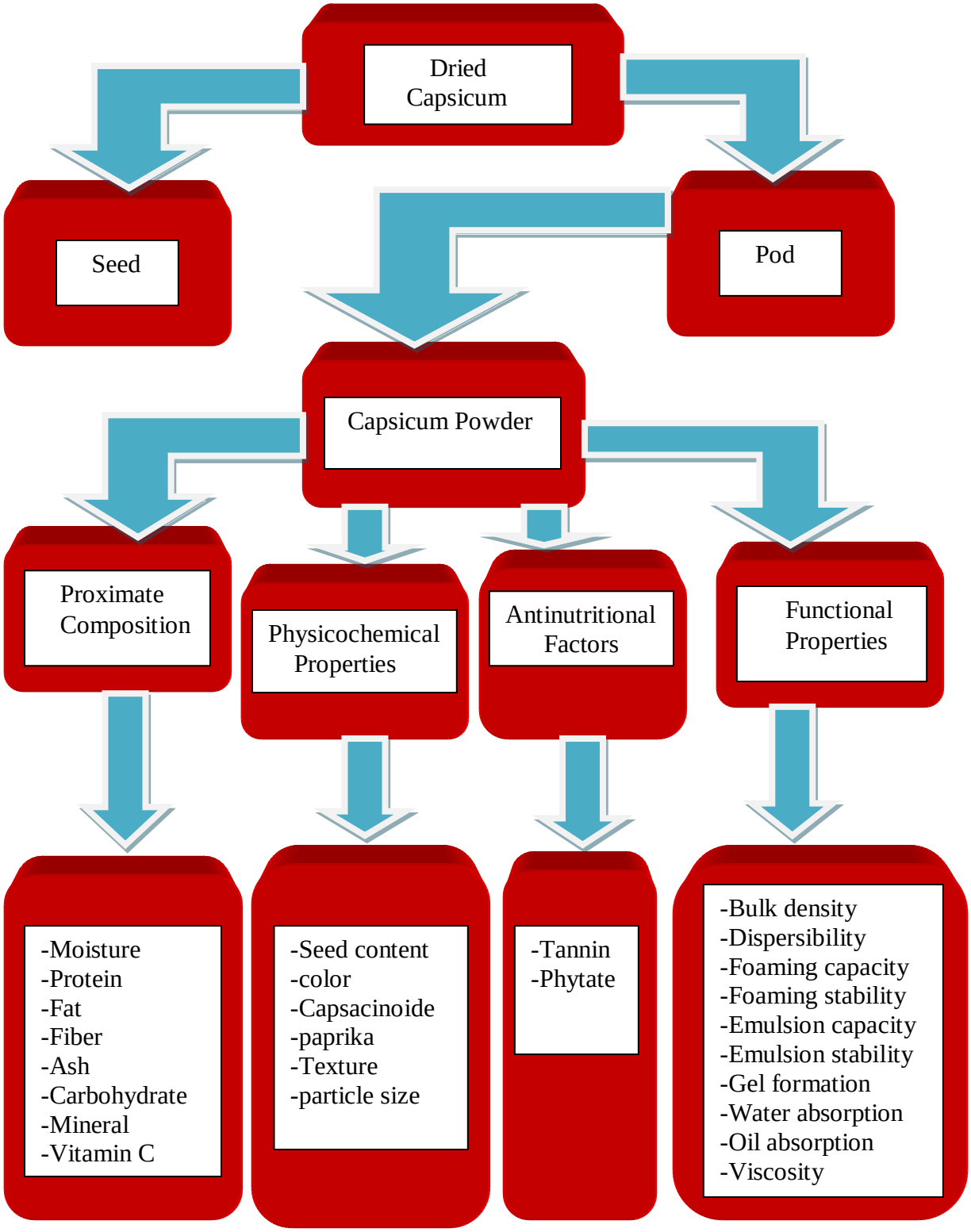


Figure 3.7 Flow chart of the methodology

3.3 Methods of Analysis

3.3.1 Proximate Composition

❖ **Determination of Moisture Content**

Moisture contents of the raw capsicum flour samples were determined according to AOAC (2000), using the official method 925.05.

The dishes used for the moisture determination were dried at 130^0c for 1 hr in Memmert drying oven of model 40050 and placed in desiccators for about 30 min. The mass of each dishes was measured (M_1) and about 5 g of the sample was weighed in to each of the dishes (M_2). The sample was then mixed thoroughly and dried at 100^0c for 6 hr. After drying is completed, the mass was measured (M_3). The moisture content was calculated from the equation:

$$\text{Moisture}(\%\,{}^{w}/_{w}) = \frac{M_2 - M_3}{M_2 - M_1} \times 100$$

M_1=mass of the dish, M_2=mass of the dish and the sample before drying, and M_3=mass of the dish and the sample after drying

❖ **Determination of Crude Protein Content**

Protein content of the raw capsicum flour samples were determined according to AOAC (2000), using the official method 979.09.

About 0.5 g of capsicum powder was weighed by Adventurer analytical balance of model AR2140 and added to the digestion flask. Then 6 mL of acid mixture (concentrated orthophospsoric acid and concentrated sulphuric acid) and 3.5 mL of 30% hydrogen peroxide solution were added in to the digestion flask step by step. The tubes were shaked until the violet reaction was disappred. About 3 g of the catalyst mixture made of 0.5 g of selenium and 100 g of potassium sulphate was added in to the digestion flask. The solution was then

digested at 370^0c for 1 hr by Tecator digester of model 722. After digestion was completed, the content in the flask was diluted by water and 40% sodium hydroxide was added to neutralize the acid and to make the solution slightly alkaline.

$$NH_4SO_4 + 2NaOH \Rightarrow 2NH_3 + 2H_2O + Na_2SO_4$$

The ammonia was then distilled into receiving flask that consisted solution of excess 4% boric acid solution for reaction with ammonia. The borate ion was formed as the result of the reaction of the boric acid and the ammonia and this was titrated with standard acid (0.1N sulphuric acid solution).

$$NH_3 + H_3BO_3 \text{ (boric acid)} \Rightarrow NH4^+ + H_2BO_3^- \text{(borate ion)}$$

The dilution, distillation and titration of the digested sample were done by using kejeltic analyzer unit.

Figure 3.8 kejeltic analyzer unit

The nitrogen content was calculated from the equation:

$$\text{Nitrogen } (\%\,{}^{W}/_{W}) = \frac{(V_2 - V_1)\times 14}{W} \times 100$$

Where V_1= volume (mL) standard H_2SO_4 solution used in the titration of the blank, V_2= volume (mL) standard H_2SO_4 solution used in the titration of the sample, W= sample weight and 14 is the molecular weight of nitrogen.

The protein content was calculated from the equation:

$$\text{Protein content } (\%^{W}/_{W}) = 6.25 \times \%N$$

❖ **Determination of Crude Fat (Oleoresin) Content**

Crude fat content of the raw capsicum flour samples were determined according to AOAC (2000), using the official method 4.5.01.

The flasks used for the extraction were cleaned by placing them in Memmert drying oven of model 40050 at 92^0c for 1 hr and cooled in desiccators. The masses of the cooled flasks were measured by Adventurer analytical balance of model AR2140 (M_1). About 2 g of the capsicum powder was weighed in to each of the thimbles lined with cotton at their bottom. The thimbles with there sample content were placed in to the Soxtec soxhlet extraction apparatus of model 2055. 70 mL of diethyl ether was added in to each flask used for the extraction. The extraction process was done for about 4 hr and then after the flasks with there contents were removed from the soxhlet and placed in drying oven at 92^0c for 1 hr .The flasks were then placed in desiccators for 30 min. The masses of each flask together with its fat contents were measured (M_2).

The crude fat content was calculated from the equation:

$$\text{Lipid } (\%^{W}/_{W}) = \frac{M_2 - M_1}{W} \times 100$$

Where M_2=mass of flask and lipid extracted and M_1=mass of dried flask and W=sample weight.

Figure 3.9 Soxtec Soxhlet fat extraction apparatus

❖ **Determination of Crude Fiber Content**

Crude fiber content of the raw capsicum flour samples were determined according to AOAC (2000), using the official method 920.169.

About1.6 g of the sample was weighed in each of 600 mL beaker. 200 mL of 1.25% sulfuric acid solution was added to each beaker and allowed to boil for 30 min by rotating and stirring periodically. During boiling the level was kept constant by addition of hot distilled water. After 30 min 20 mL of 28% potassium hydroxide solution was added in to each beaker and again allowed to boil for another 30 min. The level was still kept constant by addition of hot distilled water. Then after 30 min the solution found in each of the beaker was filtered through crucibles containing sand by placing each of them on Buchner funnel fitted with No.9 rubber stopper. During filtration the sample was washed with hot distilled water. The final residue was washed with 1% sulphuric acid solution, hot distilled water, 1% sodium hydroxide solution and finally with acetone. Each of the crucibles with their contents was dried for 2 hr at about 130^0c and cooled in desiccators and weighed (M_1). Then again they were ashed for 30

min at 550^0c in furnace and were cooled in desiccators. Finally the mass of each crucible was weighed (M_2).

The crude fiber was calculated from the equation:

$$\text{Crude fiber } (\%\,{}^{W}/_{W}) = \frac{M_2 - M_1}{W} \times 100$$

Where M_1=mass of the crucible, the sand and wet residue, M_2=mass of the crucible and the sand and W= sample weight.

❖ **Determination of Total Ash Content**

Total ash content of the raw capsicum flour samples were determined according to AOAC (2000), using the official method 941.12.

The crucibles used for the analysis were cleaned by drying at 120^0c in a Memmert drying oven of model 40050 and ignited at 550^0c in furnace for 3 hr. Then the crucibles were removed from furnace and cooled in desiccators .The mass of each of the crucibles was measured by Adventurer analytical balance of model AR2140 (M_1) and about 2.5 g of capsicum powder was being weighed in to each crucibles (M_2).The crucibles were dried at 120^0c for one hour on a Wagtech hot plate of model ST 15. The crucibles were then placed in a furnace at about 550^0c for 1 hr. After one hour the crucibles were removed from the furnace, cooled, 5 drops of distilled water was added to each of the crucible and placed in the furnace at 550^0c for 30 min. After that crucibles were again removed from the furnace, allowed to cool and 5 drops of distilled water and nitric acid were added to each of the crucible. Then the crucibles once again were inserted in to the furnace until they were become free from carbon and the residue appears grayish white. Then they were then removed from the furnace and placed in desiccators. Finally the mass each crucible was weighed as (M_3).

The total ash was calculated from the equation:

$$\text{Ash}(\%\,{}^{W}/_{W}) = \frac{M_3 - M_1}{M_2 - M_1} \times 100$$

Where M_1=mass of the dried dish, M_2=mass of the dish and the sample, M_3=mass of the dish and the sample

❖ **Determination of Carbohydrate Content**

The carbohydrate was calculated by difference with the exclusion of crude fiber.

$$\text{Utilizable carbohydrate}(\%\,{}^{W}/_{W}) = \text{Total carbohydrate} - \text{Crude fiber}$$

❖ **Mineral analysis**

Total mineral content of the raw capsicum flour samples were determined according to Osborne and Voogt (1978) by using Buck atomic absorbtion spectrophotometer (AAS) of model 201 VGP.

7 mL of 6N hydrochloric acid was added to each of the ash obtained previously and allowed to digest on a hot plate for 1 hr. Then 5 mL of 3N hydrochloric acid was added to each of the ashes and allowed to boil on a Wagtech hot plate. Each of the digests was then cooled and filtered in to 50 mL long neck round bottom flask using the Whatman filter paper (42mm). Then 2.5 mL of lanthanum chloride was added and filled to the mark with distilled water prior to analysis. The Fe, Zn, Mg, Na, Cu, Mn, Ca content in the samples were determined using Buck atomic absorption spectrometer (AAS) of model 201 VGP at 248.3nm, 213.9nm, 285.2nm, 589.0nm, 324.7nm, 279.6nm and 422.7nm while K content was determined by using atomic emission spectrometer (AES) at 766.5nm wavelengths respectively using air acetylene flame. The concentration of the elements in the sample was calculated as mg/100g.

$${}^{mg}/_{100\ g} = \frac{(C_2 - C_1) \times 50}{W \times 10} \times 100$$

Where C_1= concentration of the mineral in the blank solution, C_2= concentration of the mineral in the sample solution and W= sample weight.

Figure 3.10 Atomic Absorption Spectrophotometer

❖ **Determination of Vitamin C Content**

Vitamin C contents of the raw capsicum flour samples were determined according to Anne Marie (2001).

Iodine solution important for the analysis was prepared in a 400 mL beaker by dissolving about 5 g potassium iodide (KI) and 0.268 g potassium iodate (KIO3) with 200 mL of distilled water. 30 mL of 3M sulfuric acid was added to it. Then the solution was poured into a 500 mL graduated cylinder and diluted to a final volume of 500 mL with distilled water. It was mixed thoroughly and transferred to a 600 mL beaker.

Vitamin C standard solution which was important for the analysis was also prepared by dissolving 0.250 g vitamin C in 100 mL water. The solution was then diluted to volume in 250 mL volumetric flask.

25.00 mL of vitamin C solution and 10 drops of 1 % starch were added into a 125 mL Erlenmeyer flask. The burette was rinsed twice with 5 -10 mL of iodine solution and filled. The initial burette volume was recorded. The solution was titrated until the endpoint was reached (the first sign of blue color). The final volume was recorded. This step was important for standardization of the iodine solution prepared.

The final step was titration of the sample and for that 25 mL of the sample solution obtained by dissolving 0.25 g capsicum powder in 100 mL and 10 drops of 1 % starch were added into a 125 mL Erlenmeyer flask and the iodimetric titration was done. Finally the burette reading was taken and the concentration of ascorbic acid in the sample was calculated as mg/100g.

The vitamin C content was calculated from the equation:

$$\frac{V_1}{0.25\ g} = \frac{V_2}{V_3}$$

Where V_1=volume of iodine solution that react with the standard vitamin C, V_2=volume of iodine solution that react with the sample solution and V_3=volume of vitamin C in the sample

3.3.2 Physicochemical Properties

❖ **Average Number of Seeds Per Pod and Seed Content**

Average number of seed and seed contents of the raw capsicum flour samples were determined according to ESEF (2003b).

Average number of seeds per pod was done by counting the number of seeds found in each pods used for sample preparation and taking the average. Seed content on the other hand was determined on dividing seed weight to total sample weight.

The seed content was calculated from the equation:

$$\text{Seed content} = \frac{\text{Seed weight}}{\text{Total sample weight}} \times 100$$

❖ **Determination of Color Value**

Color value of the raw capsicum flour samples were determined according to IS (1979).

About 0.1 g of oleoresin was weighed into a 100 mL volumetric flask and made up to volume with acetone. 1.00 mL of this solution was pipeted in to a second 100 mL volumetric flask and made up to volume with acetone. Using tungsten lamp source and acetone as a blank, the absorbance of the 0.01% solution of oleoresin at 458 nm was taken.

The color value was calculated from the equation:

$$\text{Color value} = \frac{(\text{Absorbance of sample} - \text{Absorbance of blank}) \times 61{,}000}{\text{Mass of oleoresin}}$$

❖ **Determination Total Capsaicinoid**

Total capsacinoid content of the raw capsicum flour samples were determined according to ISO (1994).

The capsicum sample was grinded until 1mm was obtained and the grinded sample was homogenized and about 10 g of it was weighed. 100 mL tetrahydrofuran was added to the 10 g sample and transferred to continuous extraction apparatus. The process of extraction was occurred for 8 hr and after 8 hr the solvent was evaporated to the maximum extent possible in rotary vacuum evaporator under reduced pressure in a 250 mL round bottom flask on water bath.

About 0.05 g carbon black was added to the extract so as to maintain a ratio of the order of 10 between the extract and the carbon black. 90 mL of methanol solution was added to the solution and agitated on magnetic stirrer for 40 min. The solution was allowed to stand for five minutes. The solution was filtered through membrane filter in to 100 mL volumetric flask and diluted to the mark with methanol solution (70 parts methanol and 30 parts water).

3 mL of water and 2 mL of hydrochloric acid (1M) were added in to 25 mL volumetric flask and diluted to the mark with methanol solution (70 parts methanol and 30 parts water). This solution was used as blank acid solution (A). 3 mL of water and 2 mL 1M of sodium hydroxide were added in to 25 mL volumetric flask and diluted to the mark with methanol solution (70 parts methanol and 30 parts water). This solution was used as blank alkali solution (B).

Three 25 mL volumetric flasks which were labeled as a_1, a_2, and a_3 respectively were taken and 1mL of the filtrate, 2.7 mL of water and 2 mL of 1M hydrochloric acid was added to each of the flask. The solutions were diluted to the mark with methanol solution (70 parts methanol and 30 parts water).Three 25 mL volumetric flasks which were labeled as b_1, b_2, and b_3 respectively were taken and 1ml of the filtrate, 2.7 mL of water and 2 mL of1 M sodium hydroxide were added to each of the flask. The solutions were diluted to the mark with methanol solution (70 parts methanol and 30 parts water).

The zero and the 100% absorption of the single beam spectrometer were adjusted with methanol solution (70 parts methanol and 30 parts water). The apparatus was re-adjusted by placing solution B in measuring cell. Then the absorbance of solutions from flasks a_1, a_2, and a_3 was measured respectively at wave lengths of 248 nm and 296 nm. The absorbances of solution A was measured at 248 nm and 296 nm by placing solution A in the measuring cell. Then the absorbance of solutions from flasks b_1, b_2, and b_3 was measured respectively.

The total capsacinoide was calculated from the equation:

$$W_{248}=\frac{(A_s-A_b)\times d}{314\times M} \qquad W_{296}=\frac{(\dot{A}_s-\dot{A}_b)\times d}{127\times M}$$

A_s or A'_s=absorbance of sample solution, A_b or A'_b=absorbance of blank solution, d=dilution factor (equal to 25 X100) and m=mass in gram of test portion

❖ **Determination of Paprika Content**

Paprika content of the raw capsicum flour samples were determined according to ESEF, (2003b).

The mass of an empty container was measured (M_1) and oleoresin of known mass was added to it. The oleoresin was dissolved with methanol which was 75% of the mass of the oleoresin. The solution was then stirred with magnetic stirrer for 45 minutes and allowed to stand for nine hours. The paprika was settled at the bottom while the capsaicin was dissolved in methanol and forms a solution. The solution and the paprika were separated by pouring the solution into another container. The solvent which was left in the paprika was evaporated by heating it to 30^0c and the mass of the paprika and the container was measured (M_2).

$$\text{Paprika content} = (\%\ {}^{W}/_{W})\ \frac{M_2 - M_1}{W} \times 100$$

Where M_1= mass of an empty container, M_2= mass of paprika and container and W= mass of oleoresin

❖ **Determination of Particle Size Distribution**

Particle size distribution of the raw capsicum flour samples was determined according to Bhatty (1999).

About100 g of the ground capsicum was measured and put in a sieve shaker having 1 mm, 0.425 mm and 0.075 mm sieves. The sieve shaker was allowed to shake for 5 min. After that the Particle size of each fraction was expressed as percent of the total ground capsicum.

$$\text{Particle size distribution}(\%\ {}^{W}/_{W}) = \frac{\text{Weight of capsicum in the seive}}{\text{Total weight of capsicum used}} \times 100$$

❖ **Determination of Texture**

Texture (Firmness) of the raw capsicum flour samples was determined according to Morais *et* al., 2008.

Fruit firmness was measured by automatic texture analyzer with a 6 mm plunger moving at a speed of 1 mm/s to a depth of 2 mm. Measurements were carried out at two equidistant points on the equatorial region of whole, unpeeled fruits and results expressed in Newtons per pod (N per pod).

Figure 3.11 Texture Analyzer

3.3.3 Functional Properties

❖ **Determination of Water and oil absorption capacity**

Water and oil absorption capacity of the raw capsicum flour samples were determined according to Aremu *et al.*, (2007).

About 1 g of the grounded capsicum was mixed with 10 mL of distilled water (density of 1 g/cm^3) or oil (specific gravity of 0.989) in a centrifuge tube and allowed to stand at room temperature 30^0c for 1 hr. It was then centrifuged at 200 rpm for 30 min and the supernatant was noted in a 10 mL graduated cylinder. Water and oil absorption capacity was calculated as ml of water or oil absorbed per gram of the grounded capsicum.

Water and oil absorption capacity was calculated from the equation:

$$\text{Water and oil absorbtion capacity} = 10 - V$$

Where V= volume of water or oil left unabsorbed after centrifugation.

❖ **Determination of Bulk Density**

Bulk density of the raw capsicum flour samples was determined according to Oladele and Aina, (2007).

About 50 g grounded capsicum was put in a 100 mL measuring cylinder. The cylinder was tapped continuously until a constant volume was obtained. The bulk density was calculated as weight of the grounded capsicum (g) divided by its volume (ml).

$$\text{Bulk density} = \frac{\text{weight of ground capsicum}}{\text{Volume}}$$

❖ **Determination of Foaming Capacity and Stability**

Foaming capacity and stability of the raw capsicum flour samples were determined according to Aremu *et al.*, (2007).

About 1 g of the grounded capsicum was dispersed in 50 mL distilled water. The resulting solution was vigorously whipped for 30 min in a kenwood blender and poured into a 100ml graduated cylinder. The volume before and after whipping was recorded and the foaming capacity was calculated as percentage volume increase

The foaming capacity was calculated from the equation:

$$\text{Volume increase (\%)} = \frac{\text{volume after whipping} - \text{volume before whipping}}{\text{Volume before}} \times 100$$

Foaming stability was determined as the volume of foam that will be remained after 8 hour expressed as a percentage of the initial volume**.**

The foaming stability was calculated from the equation:

$$\text{Volume of foam (\%)} = \frac{\text{volume of foam left after 8 hrs}}{\text{Intial foam volume}} \times 100$$

❖ **Determination of Emulsifying Capacity and Stability**

Emulsifying activity and stability of the raw capsicum flour samples were determined according to Aremu *et al.*, (2007).

About 1 g of sample was blended in kenwood major blender with 50 mL distilled water for 30 second at maximum speed. Executive chief oil was added in 5 mL portions with continued blending. A drop in consistency was considered to be the point at which oil addition was discontinued. The emulsion so prepared was then being allowed to stand in a graduated cylinder, and the volume of water separated after 24 hr was recorded as emulsions stability.

❖ **Determination of Geleation**

Gelations of the raw capsicum flour samples were determined according to Aremu *et al.*, (2007).

Capsicum powder was dispersed in distilled water to make 100 mL total volume and suspension of 2-12% (w/v). The mixtures were stirred and distributed into test tubes in 5 mL aliquots and evaluated for gel formation by the least concentration end point. A series of solution concentration were heated in Memmert water both at 100^0c for 30 min. After heating, the samples were cooled at 4^0c in a cooler and the strength of the coagulum was evaluated by inverting the tube. The lowest protein concentration which form a stable gel (remain in an inverted test tube) was considered to be the galation end point.

❖ **Determination of Dispersibility**

Dispersibility of the raw capsicum flour samples were determined according to Kulkrani *et al.*, (1991).

Dispersibility was measured by placing 10 g of the sample in a 100 mL stoppered measuring cylinder, adding distilled water to reach a volume of 100 mL, stirring vigorously and allowing it to settle for 3 hr. The volume of settled particles was subtracted from 100 and the difference was reported as percentage dispersibility.

❖ **Determination of Viscosity**

Viscosities of the raw capsicum flour samples were determined according to Ikujenlola and Fashakin (2005).

The extracted oleoresin was poured into the Brookfield viscometer of Model DVII and the viscosity was measured using spindle number 42 at a shear rate of 6 rpm at 27^0c. Within 2 minute, the average of the maximum and minimum viscosity reading was recorded according to the speed. The final results obtained were expressed in terms of centipoises (c.p.s).

3.3.4 Antinutritional Factors

❖ **Determination of Tannin Content**

Tannin content of the raw capsicum flour samples were determined according to Maxson and Rooney (1972).

About 2 g of capsicum powder was weighed and extracted with 10 mL of 1% HCl in methanol for 24 hrs at room temperature with mechanical shaking. Then after centrifuged at 1000 rpm for 5 min. 1 mL of the supernatant of the centrifuged solution was mixed with 5ml of vanillin HCl reagent prepared by combining equal volume of 8% HCl in Methanol and 4% vanillin in methanol. The absorbance was read at 500 nm after 20 min. A stock D.catechin solution was used as the standard values of tannin. 20 mg D. catchin was weighed and dissolved in 100 mL 1% HCl methanol. Then 0, 0.2, 0.4, 0.6, 0.8 and 1mL stock was taken and diluted with 5 mL 1% HCl in Methanol. The absorbance was again read at 500 nm using Beckman UV-Vis spectrophotometer model Du-64.

The tannin content was calculated from the equation:

$$mg/g = \frac{\text{Absorbance} - \text{Intercept}}{\text{Slope} \times \text{Density} \times \text{Weight of sample}}$$

❖ **Determination of Phytate Content**

Pytate content of the raw capsicum flour samples were determined according to Latta and Eskin (1980).

About 2 g of samples were extracted with 10 mL 2.4% HCl in mechanical shaker for 1 hr at an ambient temperature and centrifuged at 3000 rpm for 30 min. The clear supernatant was used for phytate estimation. 1 mL of Wade reagent (containing 0.03% solution of $FeCl_3.6H_2O$ and 0.3% of sulfosalicilic acid in water) was added to 3 mL of the sample solution (supernatant) and the mixture were mixed on a vortex mixer (Thermolyne model, 37600) for 5 seconds. The absorbance of the sample solutions were measured at 500 nm using Beckman UV-Vis spectrophotometer model Du-64.

A series of standard solution were prepared containing 10, 20, 30, 40, 50, 60 µg/ml of phytic acid (analytical grade sodium phytate) in 2.4% HCl. A 3 mL of standard were added into 15 mL of centrifuge tubes with 3 mL of water which were used as a zero level (blank). A 1 mL of the wade reagent was added to each test tube and the solution was mixed on a vortex mixer for 5 seconds. The mixture was centrifuged for 10 min and the absorbances of the solutions (both the sample and standard) were measured at 500 nm by using water to calibrate the spectrophotometer.

The phytate content was calculated from the equation:

$$\text{Phytic acid in } \mu g/g = \frac{\text{Absorbance} - \text{Intercept}}{\text{Slope} \times \text{Density} \times \text{Weight of sample}}$$

Figure 3.12 UV-Visible Spectrophotometer

3.4 Data Analysis

Completely randomized design was used in all the case of the experiments and the results were assessed by analysis of variance (ANOVA) using SPSS soft ware of version 15.0. All the analysis was carried out triplicate times and results were expressed as mean$\pm$ standard deviation. LSD test was used to separate means and the significant was accepted at $P \leq 0.05$.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Proximate composition

The one way analysis of variance done on the proximate results (Table 4.1) by spss version 15.0 indicated that there is no significant difference between the three varieties in moisture, crude fiber, crude ash and carbohydrate contents at 95% significant level because the p values calculated 0.056, 0.134, 0.353 and 0.114 respectively were grater than 0.05. On the other hand significant difference were observed between the three varieties in crude protein and fat content at 95% confidence level because the p values calculated 0.02 and 0.011 were smaller than the 0.05.

Since there is no significant difference between the three varieties in moisture, crude fiber, crude ash and carbohydrate contents as the above analysis of variance indicated, the new variety, Oda haro, was obtained to have comparable moisture, crude fiber, crude ash and carbohydrate contents with that of Marako fana and Oda haro. So, consumption of any one of the varieties will give the same amount of moisture, crude fiber, crude ash and carbohydrate like the other two.

On the other hand as the above analysis of variance indicated significant difference was observed between the three varieties in crude protein and fat contents and hence to identify specifically between which varieties the significant difference exist LSD analysis was done. This analysis indicated that the significant difference exists between Marako fana and the other varieties while no significant difference was obtained between Oda haro and Bako local. So, Marako was found to have higher protein and fat content compared to Oda haro and Bako local while the later two varieties were found to have comparable protein and fat (oleoresin)

contents. Hence this would make the new variety, Oda haro, not to be preferable for large scale production of oleoresin because of the low oleoresin content compared to Marako fana.

Table 4.1 Proximate analysis result of capsicum varieties on wet weight basis (g/100g)

Parameters	Marako fana	Bako local	Oda haro
Moisture	9.156 ± 0.053[a]	9.043 ± 0.160[a]	8.744 ± 0.053[a]
Crude protein	11.809 ± 0.075[a]	8.728 ± 0.356[b]	9.210 ± 0.173[b]
Crude fiber	27.264 ± 0.178[a]	25.966 ± 1.308[a]	28.566 ± 0.805[a]
Crude fat (oleoresin)	11.162 ± 0.235[a]	9.518 ± 0.015[b]	9.147 ± 0.433[b]
Total ash	5.268 ± 0.643[a]	7.267 ± 0.043[a]	7.264 ± 2.210[a]
Carbohydrate	35.342 ± 0.564[a]	39.480 ± 0.851[a]	37.069 ± 2.065[a]

(Mean of the triplicate ± standard deviation) values with different letters across the row are significantly different at 95% confidence level.

As it can be seen from FAO food composition table (Table 2.10) dried peppers (*Capsicum annum)* contain 13.4% moisture, 12.8% protein, 11.9% fat, 56.2% carbohydrate, 22.5% fiber, and 5.7% ash. When each of the proximate results of the three capsicum varieties compared with that of the above using independent sample t-test, significant difference was found for each proximate result because the p values for each were found to be less than 0.05. Hence Marako fana was found to be smaller in moisture, protein, fat, carbohydrate and ash and

higher in crude fiber compared to the above dried pepper results. Bako local and Oda haro on the other hand obtained to be smaller in moisture, protein, fat and carbohydrate but higher in ash and crude fiber. This suggests that all the three varieties are good sources of crude fiber as compared to the above dried peppers (*Capsicum annum*) while Bako local and Oda haro are also good sources of ash.

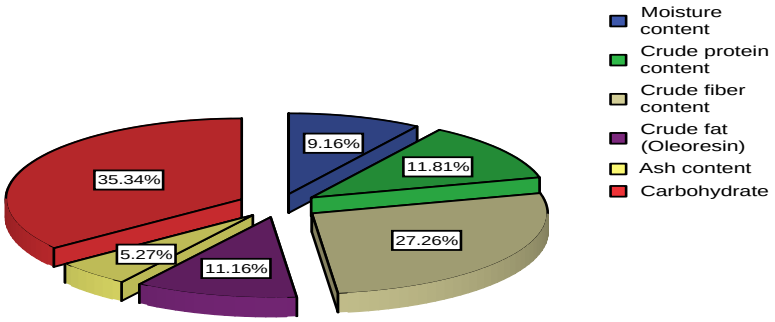


Figure 4.1 Proximate composition of Marako fana

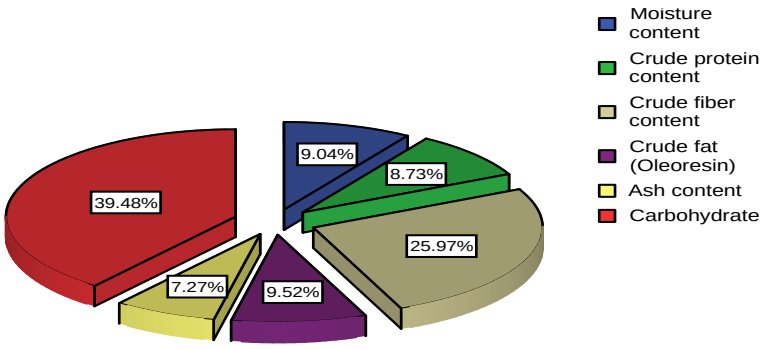


Figure 4.2 Proximate composition of Bako local

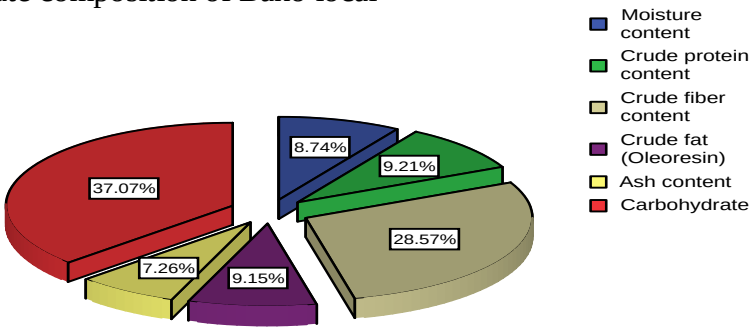


Figure 4.3 Proximate composition of Oda haro

From Table.4.1 and the pie chart, the carbohydrate and crude fiber contents of all the three varieties were found to be larger than the other proximate components. This again suggests that the three capsicum varieties can serve as a source of crude fiber.

In case of mineral analysis the potassium, magnesium, sodium, calcium, iron, zinc, copper and manganese contents of Marako fana were found to be 1.685, 8.703, 13.778, 27.156, 7.236, 3.898, 0.326 and 0.776 mg/100g while that of Bako local were found to be 1.670, 8.492, 13.966, 38.205, 6.876, 3.927, 0.281 and 0.653 mg/100g as it can be seen in the above table. The amounts of the above components in Oda haro were found to be 1.754, 8.800, 14.394, 54.565, 9.554, 2.817, 0.360 and 0.836 mg/100g respectively.

The one way analysis of variance done on each type of mineral result shown below indicated that there is no significant difference between the three varieties in magnesium, sodium, zinc, copper and manganese contents at 95% confidence level because the p values calculated 0.347, 0.583, 0.458, 0.684 and 0.786 respectively were larger than 0.05. On the other hand significant difference was found between the three varieties in potassium, calcium and iron at 95% confidence level because the p values calculated 0.023, 0.000 and 0.022 were found to be smaller than 0.05.

Since there is no significant difference between the three varieties in magnesium, sodium, zinc, copper and manganese contents as the above analysis of variance indicated, the new variety, Oda haro, was found to have comparable magnesium, sodium, zinc, copper and manganese contents with that of Marako fana and Bako local. So, consumption of any one of the varieties will give the same amount magnesium, sodium, zinc, copper and manganese like the other two.

On the other hand as the above analysis of variance indicated significant difference was obtained between the three varieties in potassium, calcium and iron contents and hence to

identify specifically between which varieties the significant difference exist LSD analysis was done. This analysis indicated that the significant difference was found between Oda haro and the other varieties while no significant difference was found between Marako fana and Bako local. Hence Oda haro was found to have higher contents of potassium, calcium and iron. These would make Oda haro to be nutritionally preferable compared to Marako fana and Bako local.

Table 4.2 Mineral composition of capsicum varieties on wet weight basis (mg/100g)

Parameters	Marako fana	Bako local	Oda haro
Potassium	1.685 ± 0.010 [a]	1.670 ± 0.022 [a]	1.754 ± 0.010 [b]
Magnesium	8.703 ± 0.163 [a]	8.492 ± 0.047 [a]	8.800 ± 0.260 [a]
Sodium	13.778 ± 0.914 [a]	13.966 ± 0.285 [a]	14.394 ± 0.044 [a]
Calcium	27.156 ± 0.164 [a]	38.205 ± 0.488 [a]	54.565 ± 0.117 [b]
Iron	7.236 ± 0.353 [a]	6.876 ± 0.313 [a]	9.554 ± 0.700 [b]
Zinc	3.898 ± 0.764 [a]	3.927 ± 0.341 [a]	2.817 ± 1.282 [a]
copper	0.326 ± 0.148 [a]	0.281 ± 0.006 [a]	0.360 ± 0.002 [a]
Manganese	0.776 ± 0.160 [a]	0.653 ± 0.067 [a]	0.836 ± 0.411 [a]

(Mean of the triplicate ± standard deviation) values with different letters across the row are significantly different at 95% confidence level.

The analysis indicated that sodium and calcium were found in higher amount in all the three varieties while copper and manganese found in very small amount compared to others. Sodium, potassium, calcium, magnesium, iron, zinc, copper, and manganese content range for capsicums grown in Tenerife Island are found to be 0.5-5.00, 177-260, 9-12, 10-14, 0.4-0.75, 0.12-0.26, 0.0065-0.104 and 0.10-0.125 respectively (Rubio *et al.*, 2002). Sodium, calcium, iron, zinc, copper and manganese content of the three capsicum varieties were found to be

above the range of capsicums grown Tenerife Island while potassium and magnesium were found to be bellow.

Table 4.3 Vitamin C analysis result (mg/100g)

Parameters	Marako fana	Bako local	Oda haro
Vitamin C	89.431 ± 8.130 [a]	84.011 ± 4.694 [a]	84.818 ± 5.906 [a]

(Mean of the triplicate ± standard deviation) values with different letters across the row are significantly different at 95% confidence level.

The vitamin C content of Marko fana was found to be 89.431 while that of Bako local was found to be 84.011 as shown in vitamin C analysis result (Table 4.3). The amount of vitamin C in Oda haro on the other hand found to be 84.818.

The one way analysis of variance done on the vitamin C result indicated that there is no significant difference between the three varieties in there vitamin C content. Hence the new variety was found to have comparable vitamin C content with that of Marako fana and Bako local. So, consumption of any one of the varieties will give the same amount of vitamin C content like the other two.

From FAO Food composition table (Table 2.10), the vitamin C content of dried *Capsicum frutescens* and *Capsicum annum* are found to be 12 and 93 mg/100g. When the above result of *Capsicum frutescens* and *Capsicum annum* compared with that of the three capsicum varieties using independent sample t-test, significant differences were found because p values are less than 0.05. Hence the three varieties found to have higher vitamin C content as compared to the *Capsicum frutescens* but smaller as compared to the *Capsicum annum*.

4.2 Physicochemical properties

The analysis of variance done on physicochemical properties results (Table 4.4) indicated that there is no significant difference between the three varieties in capsiacinoide content at 95%

confidence level because the p value calculated 0.607 were found to be greater than 0.05 while significant difference is found between the three varieties in average seed number per pod, seed content, color, paprika content and texture at 95% confidence level because the p values calculated were found to be smaller than 0.05.

As the above analysis of variance indicated, Oda haro was found to have comparable capsiacinoide content with that of Marako fana and Bako local. Hence all the three varieties are equally preferable for their pungency. The capsiacinoide content of dried Jalapeno capsicum annum fruit is 2.63% (Contreras and Yahia, 2000) while that of fresh red pepper (capsicum annum) cultivar of Spain is 0.03% (kirschbaum *et al.*, 2002). When the capsiacinoide contents of the three capsicum cultivars compared with that of above the, it was found out that three capsicum varieties do have medium capsiacinoide content.

As the above analysis of variance indicated significant difference was found between the three varieties in average seed number per pod, seed content, color, paprika content and texture and hence to identify specifically between which varieties the difference exist LSD analysis was done. This analysis indicated that in case of average number of seed per pod and seed content all the three varieties are significantly different. So, Marako fana was found to have the highest average number of seed per pod and seed content while Oda haro was found to have the lowest average number of seed per pod and Bako local was found to have the lowest seed content.

In case of color, Marako fana was obtained to be significantly different from Oda haro and Bako local while the later two was not. So, Marako fana have the highest color value while Oda haro and Bako local the lowest. Hence the new variety, Oda haro, is not preferable as a high coloring agent in foods compared to Marko fana. Even in industries for production of high food coloring agent, Oda haro is not preferable compared to Marako fana.

Table 4.4 Physicochemical properties analysis result

Parameters	Marako fana	Bako local	Oda haro
Average number of seeds per pod	135.000 ±14.411[a]	125.000 ± 14.554[b]	107.000 ± 8.300[c]
Seed content (%W/W)	46.000 ± 0.000[a]	38.983 ± 0.000[b]	43.600 ± 0.000[c]
Color value (ICU)	648331 ± 31673[a]	520687 ± 5272[b]	478085 ± 369[b]
Capsiacinoide content (%W/W)	0.217 ± 0.092[a]	0.174 ± 0.007[a]	0.161 ± 0.075[a]
Paprika content (% W/W)	0.034 ± 0.004[a]	0.022 ± 0.002[b]	0.021 ± 0.007[b]
Texture (N/pod)	13.96 ± 1.13[a]	4.66 ± 1.136[b]	4.21 ± 0.015[b]
Particle size distribution (%W/W) 1 mm sieve	0.484 ± 0.018[a]	0.042 ± 0.001[b]	0.432 ± 0.016[c]
Particle size distribution (%W/W) O.425 mm sieve	63.764 ± 0.477[a]	75.062 ± 1.434[b]	80.091 ± 0.079[c]
Particle size distribution (%W/W) 0.075 mm sieve	33.239 ± 1.446[a]	24.311 ± 0.4598[b]	17.660 ±0.1640[a]

(Mean of the triplicate ± standard deviation) values with different letters across the row are significantly different at 95% confidence level.

In terms of texture (firmness of the pod) Marako fana was found to have the highest firmness while Oda haro and Bako local have the lowest. The firmness of Bako local and Oda haro was found to be comparable. So, this indicates incase of storage, transportation and other Marako fana's resistance for mechanical damage is higher than Oda haro and Bako local.

The firmness of red pepper variety (Capsicum annum) is found to be 4.42N (Mcfeeters *et al.*, 2004). When the firmness of the three capsicum variety is compared to that of the above result, Marako fana and Bako local were found to have higher firmness while Oda haro was found to have the lower.

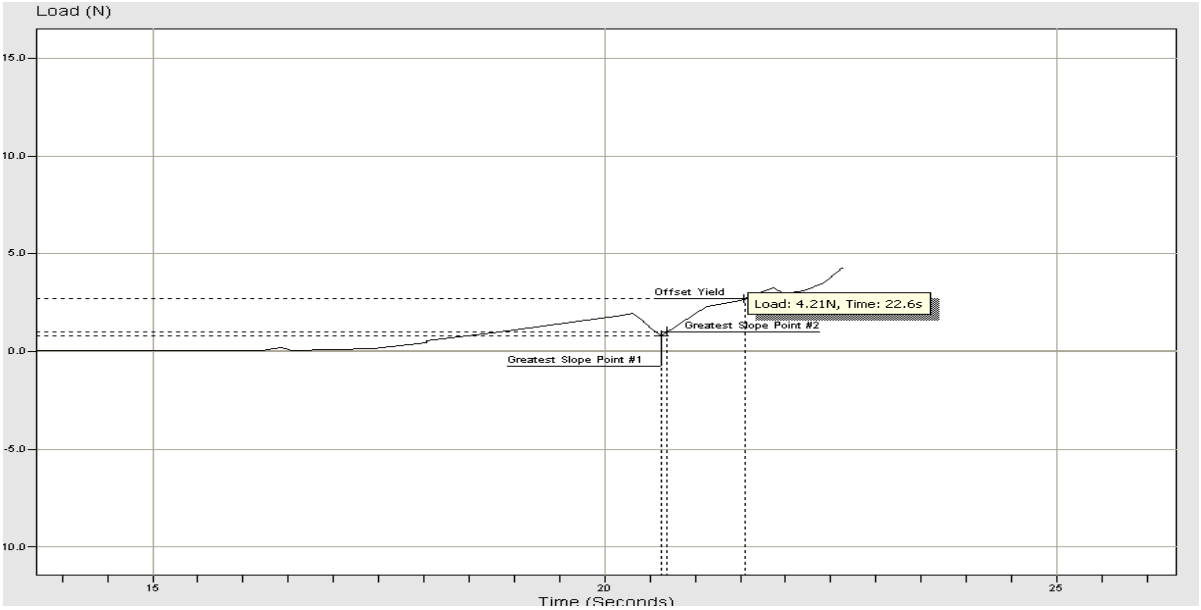


Figure 4.4 Firmness of Oda haro

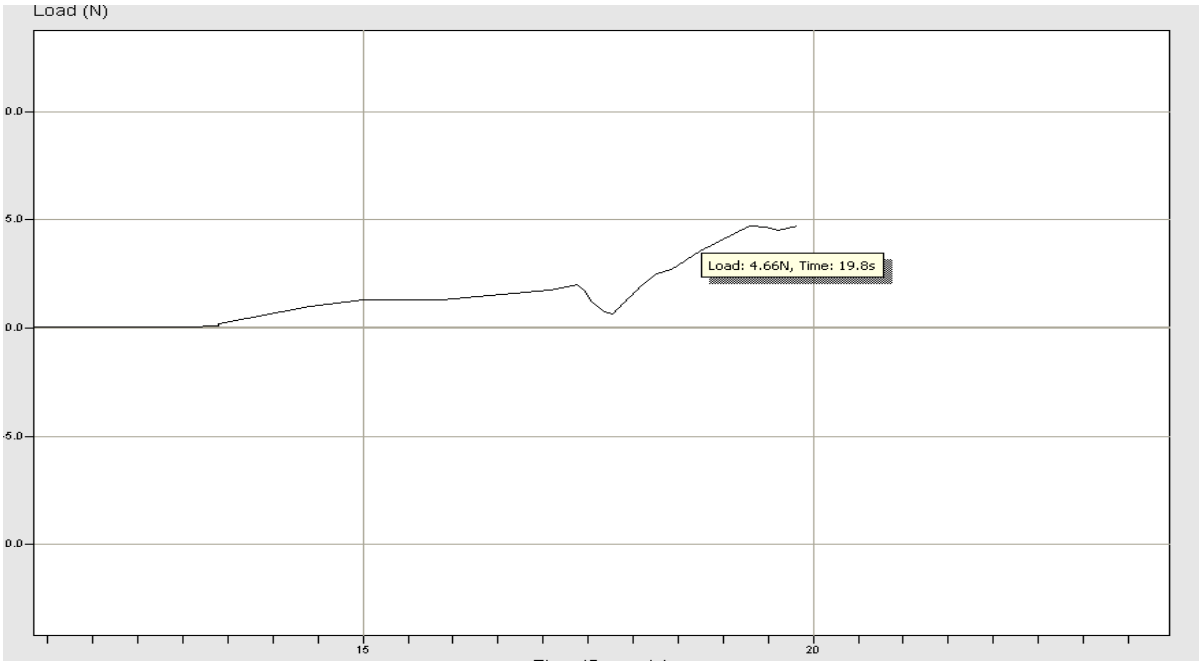


Figure 4.5 Firmness of Bako local

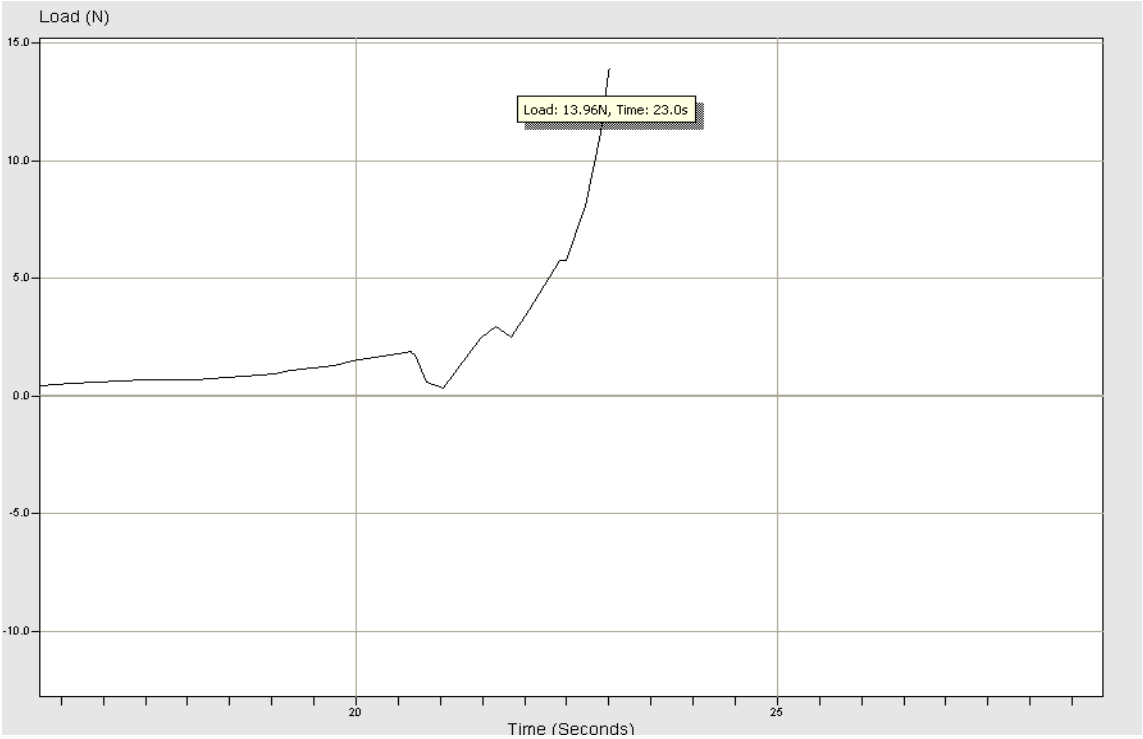


Figure 4.6 Firmness of Marako fana

The firmness of other red pepper variety (Capsicum annum) is found to be 5.34N (Van and Roozen, 1994). When the firmness of the three capsicum variety is compared to that of the above result, Marako fana was found to have higher firmness while Bako local and Oda haro were found to have the lower.

In the above figures of firmness in all the three varieties the graphs have almost parabola shape. The reason for this may be as plunger goes down and touch the pod for first time the force of resistance increases but immediately the force decrease because the plunger penetrate the pod. But finally, after penetration the plunger comes in contact with the placenta which is hard compared to the pod and result in the increment of the force.

The analysis of variance done on particle size distribution result indicated that there is significant difference between the three varieties in particle size distribution in general. In case of 1mm sieve significant difference was found between all the varieties in the parentage of

particles found in this sieve. Significant difference also found between the three varieties in the percentage of particles found in 0.425 mm sieve. The percentage of particles found in 0.075mm sieve in the case of the three varieties was also found to be significantly different.

Incase of 1mm sieve Marako fana was found to have the highest amount of particles in this sieve While Bako local was found to have the lowest. In 0.425 mm sieve Oda haro found to have the highest amount of particle in this sieve while Marako was found to have the lowest. In the last sieve Marako fana was found to have the highest while Oda haro do have the lowest.

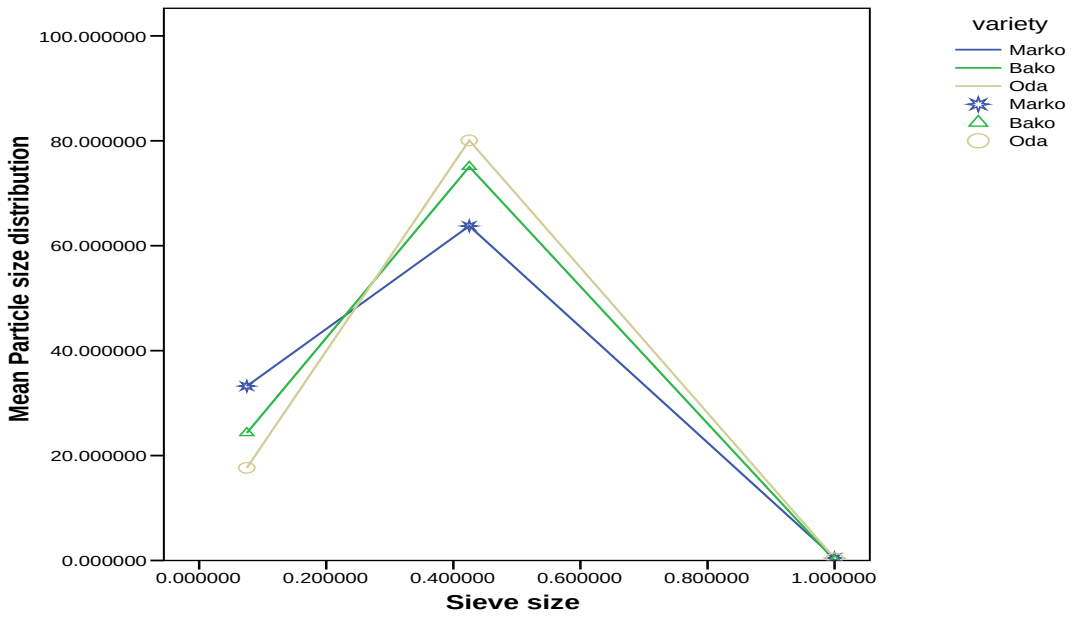


Figure 4.7 Particle size distribution and sieve size for the three capsicum varieties

From Figure 4.7 most of the particles of all the three varieties in general were found to have particle size of 0.425 mm even though the amount of Marako fana particles having this particle size were found to be smaller than the others. This may be due to the structure of the pod and the nature of grinding material. So, this indicate that the leaching out tendency of

minerals and other components of Marako fana is very less compared to the other two capsicum verities during making aqueous foods formulations.

4.3 Functional properties

Table 4.5 Functional properties analysis result

Parameters	Marako fana	Bako local	Oda haro
Bulk density(g/ml)	0.482 ± 0.009[a]	0.499 ± 0.003[b]	0.439 ± 0.008[c]
Dispersibility (%)	47.000 ± 1.000[a]	41.333 ± 0.577[b]	43.667 ± 1.155[b]
Foaming capacity (%)	1.837 ± 0.232[a]	0.922 ± 0.119[b]	2.048 ± 0.113[a]
Foaming stability (%)	10.918 ± 2.504[a]	19.333 ± 1.155[b]	9.727 ± 2.010[a]
Emulsion capacity (ml/g)	33.000 ± 1.732[a]	34.333 ± 0.577[a]	35.000 ± 1.000[a]
Emulsion stability (ml)	40.333 ± 0.577[a]	34.667 ± 0.577[b]	42.333 ± 1.528[c]
Gel formation (%w/v)	8.667 ± 0.577[a]	9.667 ± 0.577[b]	10.333 ± 0.577[b]
Water absorption(ml/g)	1.600 ± 0.707[a]	1.100 ± 1.414[a]	1.500 ± 0.000[a]
Oil absorption(ml/g)	1.000 ± 0.707[a]	1.400 ± 0.000[a]	1.450 ± 0.071[a]
Viscosity (c.p.s)	226.850± 7.705[a]	205.750± 2.475[a]	209.650± 5.162[a]

(Mean of the triplicate ± standard deviation) values with different letters across the row are significantly different at 95% confidence level.

The one way analysis of variance done on the functional properties results (Table 4.5) indicated that there is no significant difference between the three varieties in emulsion capacity, water absorption capacity and oil absorption capacity of the grinded sample and viscosity of their oleoresin at 95% confidence level because the p values calculated 0.197, 0.524, 0.524 and 0.061 respectively were found to be larger than 0.05 while significant difference was found between the three varieties in bulk density, dispersibility, foaming capacity, foaming stability, emulsion stability and gel formation at 95% confidence level

because the p values calculated 0.000,0.001, 0.000, 0.002, 0.000 and 0.003 were found to be greater than 0.05.

Since there is no significant difference between the three varieties in emulsion capacity, water absorption capacity and oil absorption capacity of the grinded sample and viscosity of their oleoresin as the above analysis of variance indicated, Oda haro powder was found to have comparable emulsion capacity, water absorption capacity and oil absorption capacity with that of Marako fana and Bako local. The viscosity of oleoresin of Oda haro was also found to be comparable with the other two varieties.

An oil-in-water emulsion is a two-phase system in which the hydrophobic phase (oil droplets) is surrounded by a continuous aqueous phase. So, the amount of oil phase surrounded by continuous aqueous phase was found to be almost equal in the three varieties.

The emulsion capacity of jack fruit flour is 7.0ml/g (Odoemelam, 2005) which is very small compared to the three varieties of capsicum. The emulsion capacity of the three varieties is very high compared to the jack fruit flour and other flours. This indicate that the aqueous solution of all the three capsicum samples do have high capacity to mix with oil parts. Hence the three capsicum varieties can be used in aqueous-oil food formulations.

Water absorption capacity describes flour – water association ability under limited water supply. It gives an indication of the amount of water available for gelatinization. Water binding capacity is a useful indication of whether flour or isolates can be incorporated into aqueous food formulations.

The water absorption capacity of jack fruit flour is 2.3ml/g (Odoemelam, 2005) while that of water yam variety is found to have 3.65 ml/g (Udensi *et al.*, 2008). The flour of tigernut variety is also found to have 1.37 ml/g water absorption capacity (Oladele and Aina, 2007). The water absorption capacity of the three varieties of capsicum was found to be less than jack

fruit flour and water yam variety and grater than tigernut variety. So, all the three varieties of capsicum have medium water absorption capacity. Hence the powder of all the three varieties of capsicum can be incorporated in aqueous food formulations.

Oil absorption is an important property in food formulations because fats improve the flavor and mouth feel of foods. The oil absorption capacity of jack fruit flour is 2.8ml/g (Odoemelam, 2005) while that of tigernut flour is found to have 1.13ml/g (Oladele and Aina, 2007). The oil absorption capacity of the three varieties of capsicum was found to be less than jack fruit flour and almost equal to tigernut variety. Hence the three varieties medium oil absorption capacity and their ability of improving flavor and mouth feel of food is medium.

The viscosity values of the three varieties on the other hand indicate that the oleoresin is not highly viscose compared to highly viscose liquids which do have 1000 c.p.s and above that.

As the above analysis of variance indicated, significant difference was found between the three varieties in bulk density, dispersibility, foaming capacity, foaming stability, emulsion stability, gel formation, water absorption capacity and oil absorption capacity and hence LSD analysis was done to identify specifically between which varieties the difference exist. This analysis indicated that all the three varieties were significantly different in case of bulk density, dispersibility and emulsion stability while Bako local was found to be significantly different from the rest two varieties in case of foaming capacity and stability. In case of gel formation significant difference was found between Marako fana and the rest two varieties.

Bulk density is a measure of heaviness of a flour sample. It gives an indication of the relative volume of packaging material required. Generally, higher bulk density is desirable for the greater ease of dispersibility and reduction of paste thickness. Bako local was found to have the highest bulk density while Oda haro was found to have the lowest. So, Bako local powder was found to have high dispersibility in water and need lower volume packaging material

compared to the other two varieties. Oda haro on the other hand found to have low dispersibility and need higher volume packaging material compared to the other two varieties. Wheat flour has bulk density of 0.57g/ml (Ade-ornowaye *et al.*, 2008) while that of jack fruit flour is 0.61g/ml (Odoemelam, 2005). On the other hand water yam variety is found to have 0.64 g/ml (Udensi *et al.*, 2008). When the bulk densities values of the capsicum varieties compared with that of above bulk densities of the different flours, the bulk density of the capsicum varieties was found to be lower. Hence the flours of the three capsicum varieties need higher packaging and have lower dispersibility compared to the above varieties.

The dispersibility of a mixture in water indicates its re constitutiability. The higher the dispersibility, the better it would be. Marako fana was found to have highest dispersibility while Bako local was found to have the lowest. So, Marako fana powder was found to have higher re constitutiability in water compared to the other two varieties while Bako local have the lowest. The dispersibilites of sorghum malt flour based weaning foods powder are from 63-79% (kulkarni *et al.*, 1991). The three capsicum varieties have lower dispersibility compared to the sorghum malt. Hence the capacity of the three capsicum varieties to reconstitute to form a paste is lower compared to the sorghum malt.

It is assumed that, once an oil-in-water emulsion is formed, the droplets in the emulsion are stabilized by a protein film at the interface. In this respect the presence of soluble proteins favored the emulsion stability. Oda haro was found to be the one which do have the highest emulsion stability while Bako local was found to be the one which do have the lowest. Even though Marako fana was found to have high crude protein content, the emulsion stability is not the highest. This may be due to the fact that the amount of soluble protein which stabilized the emulsion is small compared to Oda haro. Hence aqueous-oil food formulations can stay

homogenized in a better manner in the presence of Oda haro compared to Marako fana and Bako local.

Ibo Ekona, Country Ekona, Country Ngdere, Sosso Chad and Kwanfre Ngdere varieties of taro found to have emulsion stability of 25.87, 35.08, 42.52, 41.38, 41.72 and 0.012 ml/100ml (Njintang *et al.*, 2007) which are very small compared the three varieties of capsicum. Hence emulsion stability of three capsicum varieties are found to be higher compared to the above taro varieties. Hence aqueous-oil food formulations can stay homogenized in a better manner in the presence of the three capsicum varieties compared to the above varieties of taro.

The formability of flours has been shown to be related to the amount of native protein. Native protein gives higher foam stability than the denatured protein. It is related to the amount of solubilized protein. Bako local was found to have the lowest foaming capacity compared to the other two capsicum varieties while Oda haro and Marako fana was found to have comparable foaming capacity. The low foaming capacity of Bako local may be due to low soluble protein content. Hence Oda haro and Marako fana can be used to form foam in a food in a better manner compared to Bako local. The foaming capacity of two variety of tigernut is found be 11.07 and 10.28% (Oladele and Aina, 2007) while that of Mucuna bean protein isolate is found to have 10% (Udensi and Okoronkwo, 2006). The foaming capacity of the three capsicum varieties in general were found to be very small compared to the tigernut and Mucuna bean protein isolate. Hence the three capsicum varieties have low chance of being used in foods to formulate foam compared to the above tigernut and Mucuna bean protein isolate.

The foaming stability is related to the amount of solubilized protein. Bako local was again found to have the lowest foaming stability compared to the other two capsicum varieties while the other two was found to have comparable foaming stability. The low foaming capacity of

Bako local may be again due to low soluble protein content. Hence the foaming stability of two variety of tigernut is found be 50.6% and58.99% (Oladele and Aina, 2007). The foaming stability of the three capsicum varieties in general were found to be very small compared to the tigernut varieties. Hence the three varieties have low chance to be incorporated in foods that need stable foam formation.

The gelling capacity of flours is related to denaturation, aggregation and thermal degradation of starch. Marako fana was found to form gel at lower concentration than the other varieties while Oda haro and Bako local form gel at comparable concentration. This indicate that Marako fana be useful in food systems such as puddings and sauces in better way than Oda haro and Bako local.

The least gelation concentration of jackfruit is 16% (Odoemelam, 2005) while that of water yam variety is found to have 55% (Udensi *et al.*, 2008). Groundnut flour is also found to have the least gelation concentration of 6% to (Abulude *et al.*, 2006). The least gelation concentration of the three capsicum varieties were found to be less than that of jackfruit and water yam variety and greater than the groundnut flour. Hence the three capsicum varieties can be included in aqueous food formulations like sauces, pudding, snack cookies, milk and milk products and others.

4.4 Antinutritional Factors

In case of antinutritional factors the tannin content of Marako fana, Bako local and Oda haro were found to be 0.142, 0.164 and 0.148 mg/100g respectively while no phytate was detected in all the three varieties.

The analysis of variance done on the above result indicated that there is significant difference between the three varieties in tannin content at 95% confidence level because the p value calculated 0.001 was found to be smaller than 0.05 and hence LSD analysis was done to

identify specifically between which varieties the difference exist. The LSD analysis indicated that all the three varieties are significantly different. So, Bako local was found to have the highest tannin content while Marako fana was found to have the lowest.

Table 4.6 Antinutritional composition of the three varieties

Parameters	Marako fana	Bako local	Oda haro
Tannin (mg/100g)	0.142 ± 0.001^{a}	0.164 ± 0.002^{b}	0.148 ± 0.001^{c}
Phytate (mg/100g)	ND	ND	ND

(Mean of the triplicate $\pm$ standard deviation) values with different letters across the row are significantly different at 95% confidence level.

The tannin content of Indian bean (*Dolichos lablab L.*) is found to be 0.85 mg/100g (Ramakrishna *et al.*, 2008) while that of two pearl millet (*Pennistem glaucum L.*) cultivars, Gazira and Gadarif, are found to be 220 and 170mg/100g respectively (Eltayeb et al., 2007). When the tannin content of the three capsicum varieties compared with that of the above cultivars, significant differences were found because p values are less than 0.05. So, the tannin content of the three capsicum varieties was found to be smaller than the above cultivars. Hence, the effect of tannin on protein availability is not that much in the three capsicum varieties compared to the above cultivars. Since there is no phytate, the possibility of finding the mineral in available form is large in all the three varieties.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The over all nutritional, functional, and physicochemical properties analysis done on the three capsicum varieties, Marako fana, Bako local and Oda haro, has indicated that each variety has its own nutritional, functional and physicochemical properties which would make it to be preferable compared to the other two.

Oda haro is nutritionally preferable for higher potassium, calcium and iron need. It needs higher volume of packaging material compared to Marako fana and Bako local. It can be used in a better manner in a situation where aqueous-oil food formulations are needed to stay being homogenized. It forms foam in a food in higher amount than Bako local. But it is not preferable for large scale production of oleoresin and paprika in industries. It is not good coloring agent as compared to Marako fana. It has low dispersibility in water.

Marako fana is preferable for large scale production of oleoresin and paprika in industries. It is good coloring agent. It can not be easily damaged during storage and transportation. The minerals in it can not be easily leached out during aqueous food formation and hence important in aqueous food formulations. It forms foam in a food in higher amount than Bako local. It can be used in food systems such as puddings and sauces in better way than Oda haro and Bako local because it forms gel easily. It has low tannin and hence chance of getting protein in available form is high. It is better than the other two varieties for higher protein need. It has higher reconstitution ability in water.

Bako local needs lower volume of packaging material. It has higher dispersibility but lower reconstitution ability in water. It has high tannin content and hence the chance of getting protein in available form is small compared to Marako fana ad Bako local.

All the three varieties have equal degree of pungency and chance of getting minerals in available form is high in all the three of them. They are also comparable in amount of oil phase surrounded by continuous aqueous phase, water available for gelatinization, capacity of improving the flavor and mouth feel of foods.

5.2 Recommendations

Oleoresin paprika and oleoresin capsicum are processed products of pepper which produce foreign currency income for the country through exportation. But the new variety was found to contain small amount of oleoresin paprika and oleoresin capsicum and its color value was found to be small compared to Marko fana and hence the search for capsicum variety which gives high amount of oleoresin paprika, oleoresin capsicum and color value should be continued by scientists in the agriculture and genetic fields, nutritionist and food technologist.

In terms of analysis sensory evaluation, digestibility, extraction efficiency, causes of distraction during storage and transportation, capsicum drying technologies, milling of capsicum and shelf life of products from capsicum need further research studies.

Integration of nutritional properties with agricultural research programs needs to be consolidated and equal attention must be given to the selection of genotype (new variety) that meet consumer criteria in terms of high nutrient density, preferred color, required size and higher digestibility with less antinurient factors. Therefore stimulated interaction is needed between scientists in the agriculture and genetic fields, nutritionist, food technologist, policy makers, NGOS and private sectors (investors) in order to demonstrate the means of crop and food improvement for need of subsistence farmers, consumers and other stack holders especially in developing country like Ethiopia.

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DECLARATION

I, the undersigned, declare that this thesis is my original work and that all sources of materials used for the thesis have been correctly acknowledged.

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