Process Optimization of Oat Based Functional Chocolate and its Shelf Life Evaluation

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In

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Supervisor

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Submitted by

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CENTRE OF FOOD SCIENCE AND TECHNOLOGY

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To,

The Registrar
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Dear Sir,

It is my pleasure in forwarding the thesis entitled, **"Process Optimization of Oat Based Functional Chocolate and its Shelf Life Evaluation"** submitted by **Ms. Pooja Shukla**, I.D. No. **FST- 15252** in partial fulfillment of the requirements for the degree of **Master of Science** in **Food Science and Technology,** Institute of Agricultural Sciences, Banaras Hindu University, Varanasi and placing on record that she has completed the requisite residential requirements as contained in the ordinance of the University.

I certify that the entire scheme of investigation, presented here in, was planned and carried out solely by the candidate under my supervision and guidance. To the best of my knowledge, the data presented in the thesis are genuine and original.

Thanking You,

Yours Faithfully,

FORWARDED BY

(Er. Durga Shankar Bunkar)
Supervisor

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Process Optimization of Oat Based Functional Chocolate and its Shelf Life Evaluation

By

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Thesis submitted in partial fulfillment for the requirements for the award of degree of

MASTER OF SCIENCE IN FOOD SCIENCE AND TECHNOLOGY FROM CENTRE OF FOOD SCIENCE AND TECHNOLOGY, INSTITUTE OF AGRICULTURAL SCIENCES, BANARAS HINDU UNIVERSITY, Varanasi.

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LIST OF ABBREVIATIONS AND SYMBOLS

%	:	Percentage
µmol/ml	:	Micromole per mililiter
AACC	:	American Association for Clinical Chemistry
AOAC	:	Association of Official Analytical Chemists
BandT	:	Body and texture
CandA	:	Colour and appearance
CFU	:	Colony forming unit
CL	:	Curry leaves
DC	:	Dark chocolate
cm	:	Centimeters
Db	:	Dry basis
Fig.	:	Figure
g	:	Gram
g/g	:	Gram per gram
h	:	Hours
IS	:	Indian Standard
Kg	:	Kilograms
kg/m^2	:	Kilograms per meter square
MAP	:	Modified atmospheric packaging
Mg	:	Milligrams
Min	:	Minutes
Ml	:	Milliliter
Mm	:	Millimeter
N	:	Normality
$^{\circ}C$	:	Degree centigrade/Celsius
Nm	:	Nanometer
OOA	:	Overall acceptability
RSM	:	Response Surface Methodology
Sd.	:	Standard deviation
Sec.	:	Second
SF	:	Surface Plot
TPC	:	Total plate count
TPA	:	Texture Profile Analyzer
TS	:	Total solids
TSS	:	Total soluble solids
TVC	:	Total viable count
UV	:	Ultra violet
V/v	:	Volume by Volume
W/w	:	Weight by weight
YMC	:	Yeast and mold count
ΔT	:	Change in temperature

CHAPTER-1

Introduction

Chocolates are and widely consumed. Chocolate comprises a number of raw and processed foods produced from the seed of tropical *Theobromu cacao* tree. Chocolates have health promoting aspects and are increasingly in demand due to their taste, aroma. Different substances are being incorporated for improvement and as a part of experiment to provide textural and reological properties to the chocolate. The chocolate with higher cocoa contains higher antioxidant content (Yap, 2010).

Generally chocolate is categorized as dark, milk and white chocolate with different ratio of cocoa solid, milk fat and cocoa butter. Cocoa butter is the only fat phase in chocolate responsible for the dispersion or other constituents (Lipp and Anklam, 1998). Dark chocolate contains the highest total catechin content (53.5 mg/100g). The process of manufacturing of chocolate depends on consumer acceptance and company practices (Awua *et al*., 2002; Beckett *et al*., 2000; Whitefield *et al*., 2005).Specification of chocolate depends on its type and its intended use. It also has been observed that despite of having high sugar and lipid content, consumption of chocolate imparts beneficial effect on human health, as it contains antioxidants including favonoids such as epicatechin, catechin and procyanidins.

Chocolate is also a good source of minerals like potassium, magnesium, iron and copper (Holland et al.,1991). Cocoa is rich in poly-phenols particularly in (Flavan-3-ols) and procyanidins. The total polyphenol content of bean is estimated to be 6-8% by weight of the dry bean (Zumbe, 1998). The liavan-3-ols have been identified as the major antioxidant components of different cocoa ingredients and chocolate preparations that are useful in curing severe diseases such as cancer, heart disorders and also increases longevity of life (Osakabe, 1998; Admson, 1999; Baba, 2000). Epidemiological studies have reported a reduced risk of coronary heart disease with a high flavonoid intake (Grassi, 2005; Buijsse, 2006). Chocolates help arteries relax, widen and maintain their flexibility, which aid in lowering blood pressure (Scharffen Berger *et al*., 2007). Thompson and Manore (2009) also revealed that

studies have shown chocolate have the ability to reduce the risk of cardiovascular diseases.

Chocolate is used in the cases of migraines, circulatory benefit, aphrodisiac, muscle recovery, acne. reduces LDL cholesterol, natural, anti-depressant, prevents tooth decay, as a cancer tighter and as a stimulant (Whitefield *et al*., 2005). Chocolate has been also used in homeopathic medicines. Chocolates are solid at ambient (20-25°C) and melt at oral temperature (37°C) during consumption giving a smooth suspension of particulate solids in cocoa butter and milk fat (Beckett *et al*., 1999).

Chocolates with moisture contents or above 2% normally unacceptable as they have poor keeping qualities as well as a poor texture. The chemical structure of flavonoids suggests that they have antioxidant capacity, the ability to scavenge free radicals, and chelate redox active metal ions. He study and work was conducted with the aim of developing a nutritious and health promoting product – dark chocolate bar fortified with oats and curry leaves having various medicinal properties which is appreciable in consumption and consumer acceptance.

The oat, sometimes called the common oat, is a species of cereal grain grown for its seed, which is known by the same name. Oats are suitable for human consumption as oatmeal and rolled oats. Oats are a whole-grain cereal, known scientifically as *Avena sativa*. They are mainly grown in North America and Europe. They are a very good source of fiber, especially beta-glucan, and are high in vitamins, minerals and antioxidants. Whole oats are the only source of a unique group of antioxidants called avenanthramides, believed to have protective effects against heart disease. Due to their beneficial health effects, such as lowering blood sugar and cholesterol levels, oats have gained considerable interest as a health food..

Whole grain oats are called oat groats and are most commonly rolled or crushed into flat flakes and lightly toasted to produce oatmeal. Quick, or instant oatmeal is made up of more thinly rolled or cut oats that absorb water much more easily and therefore cook faster. Due to their beneficial health effects, oats have gained considerable interest as a health food. Carbs make up 66% of oats. Oats are very low in sugar, with only 1% coming from sucrose. About 11% of the carbs are fiber and 85% consists of starch which is the single biggest component of oats, made

up of long chains of glucose molecules. The starch in oats is different than the starch in other grains. It has a higher fat content, and a higher viscosity (ability to bind water). Three types of starches are found in oats, classified with respect to digestibility. Rapidly digested starch (7%)**,** which is quickly broken down and absorbed as glucose. Slowly digested starch (22%)**,** that is broken down and absorbed more slowly. Resistant starch (25%)**,** which functions like a type of fiber. It escapes digestion and improves gut health by feeding the friendly gut bacteria..

Oats contain almost 11% fiber, and majority of the fiber in oats is soluble, called beta-glucan. Beta-glucans are unique among fibers, as they can form a viscous (gel-like) solution at a relatively low concentration. In raw, whole oats, the amount of beta-glucan ranges from 2.3-8.5%, mostly concentrated in the oat bran. Beta-glucans are known to lower cholesterol levels and increase excretion of bile acids. They are also believed to cause a reduction in blood sugar and insulin levels after a carbohydrate-rich meal. Daily consumption of beta-glucans has been shown to lower cholesterol, especially LDL (the "bad") cholesterol, and may therefore decrease the risk of heart disease. Insoluble fibers, including lignin, cellulose and hemicellulose. Oats contain more soluble fiber than other grains, leading to slower digestion, increased satiety and suppression of appetite. Oats are a good source of quality protein, ranging from 11-17% by dry weight, which is higher than most other grains. The major protein in oats is called avenalin (80%), which is not found in any other grain, but is similar to legume proteins. A minor protein is a prolamin called avenin, which is related to gluten in wheat (23).However, pure oats are considered safe for most people with gluten intolerance. Whole oats contain more fat than most other grains, ranging from 5-9%. It consists mostly of unsaturated fatty acids (3). Oats contain high amounts of many vitamins and minerals, such as manganese, phosphorus, copper, B-vitamins, iron, selenium, magnesium and zinc. Only found in oats, avenathramides are a family of powerful antioxidants. They may reduce arterial inflammation and regulate blood pressure. Ferulic Acid is **t**he most common polyphenol antioxidant in oats and other cereal grains. Phytic Acid is most abundant in the bran, phytic acid is an antioxidant that can impair the absorption of minerals, such as iron and zinc (12, 38).Oats, as oatmeal or oat bran, can lower cholesterol levels, which should reduce the risk of heart disease.

Oats have also been claimed to lower blood pressure and reduce the risk of obesity and type 2 diabetes. Blood cholesterol is a major risk factor for heart disease, especially oxidized LDL-cholesterol. Numerous studies have shown the effectiveness of oats or oat bran in lowering blood cholesterol levels, which is mainly attributed to their beta-glucan content. Two suggested mechanisms for these cholesterol-lowering effects have been proposed. First, beta-glucan may slow the absorption of fats and cholesterol by increasing the viscosity of the digestive contents. Second, beta-glucan binds with cholesterol-rich bile acids in the intestine, produced by the liver to aid digestion. Beta-glucan then carries them down the digestive tract and eventually out of the body. Normally, bile acids are recycled (re-absorbed) in the digestive system, but beta-glucan inhibits this recycling process, leading to reduced levels of cholesterol in the body. Authorities have approved the health claim that foods containing at least 3 grams of beta-glucan per day may lower the risk of heart disease.

Beta-glucans, the soluble fibers from oats, have been tested in patients with type 2 diabetes, showing beneficial effects on blood sugar control. Modest amounts of beta-glucans from oats have been shown to moderate both glucose and insulin responses after carbohydrate-rich meals. In patients with type 2 diabetes and severe insulin resistance, a 4-week dietary intervention with oatmeal resulted in a 40% reduction in the insulin dosage needed for stabilizing blood sugar levels. Studies suggest that beta-glucans may favorably alter insulin sensitivity, delaying or preventing the onset of type 2 diabetes, but a recent review study concludes that the evidence is inconsistent. Boiled whole oats cause low glucose and insulin responses, but the responses increase significantly if the oats are ground to flour before cooking. Satiety plays an important role in energy balance. It stops eating and prevents us from eating again until hunger returns. Altered satiety signalling has been associated with obesity and type 2 diabetes. In a study ranking the satiety effect of 38 common foods, porridge (cooked oatmeal) ranked 3rd overall, and 1st among breakfast foods. Water-soluble fibers, such as beta-glucans, may increase satiety by delaying stomach emptying, increasing stomach distension and promoting the release of satiety hormones. Human trials have shown that oatmeal, rich in beta-glucans, may increase satiety and reduce appetite when compared to a ready-to-eat breakfast cereal and other types of dietary fiber. In addition to being highly satiating, oats, eaten as porridge, are

low in calories and contain plenty of fiber and other healthy nutrients, making them an excellent addition to an effective weight loss diet.

A gluten-free diet is the only solution for individuals who suffer from celiac disease, as well as for many individuals with gluten sensitivity. Oats do not contain gluten, but they contain a similar type of protein, called avenin. Clinical studies have shown that moderate or even large amounts of pure oats can be tolerated by most celiac disease patients. Oats have been shown to enhance the nutritional value of gluten-free diets, increasing both mineral and fiber intakes, and individuals usually prefer to include oats in their gluten-free diets. The biggest problem with oats in a gluten-free diet is contamination with wheat, because oats are often processed in the same facilities as other grains. Therefore, it is important for celiac patients to only eat oats that have been certified as "pure" or "gluten-free". Oats are usually well tolerated, with no adverse effects in healthy individuals. Avenin-sensitive individuals may experience adverse symptoms, similar to those of gluten intolerance, and should exclude oats from their diet. Oats may be contaminated with other grains, such as wheat, making them unsuitable for people with celiac disease (gluten intolerance) or wheat allergy. It is important for individuals allergic or intolerant to wheat, or other grain types, to buy only oats that are certified as pure from contamination. Oats are among the world's healthiest grains, a good source of many vitamins, minerals and unique plant compounds. and contain large amounts of unique soluble fibers called beta-glucans, which provide numerous health benefits. Such as lowering cholesterol, reducing blood sugar and insulin responses, relieving in constipation and improving immune function. These are also very filling, and may reduce appetite and help you eat fewer calories.

Curry leaves *(Murraya koengii*) and it belongs to the **Rutaceae** family. The plant is native to India and is are household name and find a variety of applications. The most useful parts of this plant are the leaves, root and the bark. The leaves have always been sought after for their unique flavour and usefulness in cooking, but there are also a number of health benefits that makes them highly appealing. The leaves can be dried or fried, depending on the intended use, and the fresh form is also very popular, both for cooking and herbal medicine. In Ayurvedic medicine, curry leaves are believed to have several medicinal properties such as anti-diabetic, antioxidant,

antimicrobial, anti-inflammatory, anti-carcinogenic and hepato-protective (capability to protect liver from damage) properties. The roots are used for treating body aches and the bark is used for snake bite relief. The leaves, with their vast herbal properties, are used in various local cuisines across India and other parts of Asia as flavouring agents. Curry leaves resemble 'neem' or Indian lilac and their name in most Indian languages translates to 'sweet neem'. The main nutrients found in curry leaves are carbohydrates, energy, fibre, calcium, phosphorous, iron, magnesium, copper and minerals. It also contains various vitamins like nicotinic acid and vitamin C, vitamin A, vitamin B, vitamin E, antioxidants, plant sterols, amino acids, glycosides and flavonoids. Also, nearly zero fat (0.1 g per 100 g) is found in them. Some of the other chemical constituents present in curry leaves include carbazole alkaloids. Research studies held by the Department of Home Economics at Kenmei Women's Junior College in Hyogo, Japan showed that alkaloids found in the leaves possess antioxidant properties. Carbazole alkaloids include mahanimbine, murrayanol, mahanineoenimbine, O-methylmurrayamine A, O-methylmahanine, isomahanine, bismahanine and bispyrayafoline. . Further studies conducted at the Department of Horticulture at Michigan State University suggested that these chemicals had insecticidal and antimicrobial properties as well, specifically mosquitocidal properties.

Curry leaves not just add flavor to the food , they are far more important than many people realize, and they offer a number of health benefits without the side effects as with some other medicines. Stops Diarrhea, has gastrointestinal Protection as it possess mild laxative properties. Research studies conducted by Mylarappa B. Ningappa *et al*. at Jawaharlal Nehru Center for Advanced Scientific Research, Molecular Parasitology and Protein Engineering Laboratory in Bengaluru, India have indicated that curry leaves or *Murraya koenigii* is a good source of antioxidants. The presence of various vitamins like vitamin A, vitamin B, vitamin C and vitamin E help in reducing oxidative stress and free radical scavenging activity. The leaves can be added to your curries, vegetable stews and soups. They are also available powder form. One of the biggest health benefits of curry leaves is its use in diabetes control. Research conducted by the Department of Biochemistry and Molecular Biology at University of Madras, Chennai had shown that the anti-hyperglycemic properties of the leaves were beneficial in controlling blood glucose levels in diabetic rats. The

chemical constituents found in curry leaves such as phenols are helpful in fighting cancers such as leukemia, prostate cancer and colorectal cancers. Research on these leaves at the Department of Medical Chemistry at Mejio University, Japan showed evidence of cancer fighting properties in the carbazole alkaloids extract from curry leaves. Curry leaves are also known to reduce bad LDL cholesterol level. Studies conducted at the Department of Biochemistry at the University of Kerala, India have shown that they have the potential to reduce LDL cholesterol levels. Curry leaves are believed to help in strengthening hair roots. Dry curry leaf powder mixed in oil can be applied to your hair with a quick massage. The paste from curry leaves can also be applied in cases of gray hair. Studies on the extracts of curry leaves have shown positive results in reducing the effects of chemotherapy and radiotherapy, while also offering protection against chromosomal damage, protection of bone marrow and prevention of free radicals becoming active in the body .It is also effective in fighting bacterial and fungal infections. The leaf extracts from the plant have been comparable to popular, mainstream antibiotic drugs. The tannins and carbazole alkaloids present in the leaves exhibited good hepato-protective properties. They are also helpful in protecting the liver from various diseases such as hepatitis and cirrhosis. The juice or paste of the leaves can be applied on burns, cuts, bruises, skin irritations and insect bites for quick recovery and clean healing.

Sugar is used as a natural sweetener instead of any artificial sweetener. The artificial sweeteners added into “chocolate for diabetes” are very known neurotoxins. They cause same the same hormonal imbalance from consuming other sugary foods (Yap, 2010). Powdered sugar was used and the sugar content was maintained 8 % in the product.

The product contains dark chocolate that is appreciated by a large population worldwide. It is good for health despite high fat content and incorporation of dark chocolate with oat and curry leaves powder only enhances the benefits. The oats provide fiber and mass while curry leaves impart their characteristic taste and flavour to the product. The various vitamins, minerals, antioxidants, phytosterols and proteins present in the source have promising effects in promoting health. The product is considered to be free of any health losses and side effects and effective for prevention of certain health ailments and improving skin and hair conditions.

OBJECTIVES:

1. To prepare oats and curry leaves based functional chocolate.
2. To optimize the ingredient mixture using response surface methodology.
3. To study different physico-chemical, textural, sensory and microbial properties of the functional chocolate.
4. To study the shelf-life of the product.
5. To workout the cost of production of the product.

Chapter-2

REVIEW OF LITERATURE

Oats have been cultivated for over two thousand years and originated in Europe where they were used for medicinal purposes (Butt *et al*., 2008). In the 19th century oats began to replace many breakfast cereals and today are consumed regularly for their nutritional content and healthy heart benefits (Duss and Nyberg, 2004). (Correia, 2009; Dorsey Kockler, 2011; Kromann and Green, 1980; Lehtinen *et al*., 2009; Oomah, 2003) Omega-3 fatty acid, protein and fiber in *Chia* and some other foods added aid in the prevention of cardiovascular disease, boost brain health and function, improve digestive health and help weight management due to addition of oats. Therefore, the addition of dried fruit and dark chocolate ingredients would enhance the antioxidant content. Antioxidants have been observed to prevent and lower the risk of many types of cancer by inhibiting oxidative damage caused by free radicals (Kasote, Hedge and Deshmukh, 2011; Routray and Orsat, 2011).

Oat (*Avena sativa* L.) grain belonging to the Poacae family have distinctive nutritional profile compared with other types of grain including rich protein content of higher biological value, a grc-x proportion of unsaturated fatty acids and a high dietary fiber content; both as soluble and insoluble fiber. Indeed, fiber represents magnesium, as well as a rich source of (30 RDA) of thiamine, folate, zinc and phosphorus (Ruxton Carrie, 2014).

Composition

An innovative sensory analysis technique called Free choice profiling (FCp) technique was applied to examine the analyical abilities of consumers (n 39) from two different locations, one in Alistria (Vienna) and the other in Germany (Dresden) to characterize plain chocolate reported by Thamke *et al*. (2009). They investigated cocoa content or the chocolate samples ranged from 60 10 75. The characterization of chocolate was based on cocoa concentration with respect Io mouth feel' it was melting and creamy due to high cocoa content, whereas dry, mealy and sticky due to low cocoa content.

The majority of ready to cook (RTC) breakfast foods contain large quantities of whole grains; such as wheat products and oat products. Oats are generally consumed as whole grain or bran-enriched products while wheat is mainly consumed as refined flour (Lehtinen *et al*. 2009) to cook Quick oats are often used in prepackaged oatmeal, also known as instant oatmeal, and are very popular with consumers.

Nandkarni (1976) studied that green leaves of *Murraya koenigii* are eaten raw for cure of dysentery, diarrhoea and for checking vomiting. Leaves and roots are also used traditionally as bitter, anthelmintic, analgesic, curing piles, inflammation, itching and are useful in leucoderma and blood disorders

Acetylcholinesterase inhibitory potential of a carbazole alkaloid, mahanimbine, from *Murraya koenigii* leaves was studied by Kumar *et. al*. (2010). This study is the first to reveal this activity in carbazole alkaloid mahanimbine, isolated from *Murraya koenigi*i

Effect of processing parameters.

The migration or lipid in two phase chocolate system by magnetic resonance and differential Scanning Calorimetry analysed by Walter and Cornillon *et al*. (2002). They took two different sample such as lauric acid with chocolate and peanut butter with chocolate. They studied that kinetic of fat migration and found that diffusion coefficient of lauric acid in chocolate depends upon migration time. Fat bloom formation enhanced by the fat migration in chocolate due to capillary action and related to the thermal history of sample and presence of fat in the chocolate layer Fat bloom was prevented by increased the liquid to solid ratio and this is increased by lauric acid and peanut butter.

Briones *et al*. (2006) studied the effect of surface topography on colour and gloss of chocolate samples. They examined surface for roughness, colour and image texture and gloss using laser scanning microscopy, digital vision system and gloss meter respectively. The two parameter used to characterized their surface are statistical average roughness (pm) and the area scale fractal complexity (dimensionless). ASFC (Area-Scale Fractal complexity) surface of the sand paper and

chocolate sample were highly correlated with these parameters. Surface element related to roughness were in the order of 3-l4 pm. Gloss of chocolate surface diminished exponentially as roughness increased while colour decreased linearly and image, textur, entropy and homogeneity parameter varied linearly with AFSC value

Afoakwa *et al*. (2008) reported that fat crystallisation behaviour in dark chocolate from varying particle size, distribution, yield in products from different temper regime (under, optimal and over) and their effect on mechanical properties and appearance. Particle size inversely related with texture and colour, with greatest effect noted in hardness stickiness and lightness at all temper regimes. The product hardness and stickiness increased while gloss and darkening of product surface decreased in over tempering induced fat bloom in product with consequential quality defect on texture, colour and surface gloss. Both over tempering and under tempering regime adversely affect the texture and appearance during crystallization of dark chocolate can be used to achieve the desire texture and appearance.

Afoakwa *et al.* (2009) further studied that particle size distribution and temper influence on dark chocolate fat crystallization and microscopy to establish relation with their melting properties and microstructure. Under tempered chocolate showed widened Crystal Size Distribution (CSD). Over tempering caused increased in CSD and melting properties. However, under over and optimally tempered product does not affect the fat and sugar components. Thus attainment of optimal temper regime during pre-crystallization of dark chocolate is necessary for achievement of premium quality product and avoiding defect in structure and melting character.

Chevalley *et al*. (1991) reported that soy lecithin, which is the applied most widely emulsifier in chocolate manufacturing is usually added in concentrations or 3-6 g/kg chocolate mass. The addition of 1-3 g/kg soy lecithin causes the same viscosity-reducing effect as approximately l0 times this amount of added cocoa butter, thus allowing reduction in production costs by saving cocoa butter.

Schantz *et al*. (2005) studied systematically varied blends of lecithin and PolyglycerolPolyricinoleate (PGPR) added in a concentration up to 14g/Kg on the flow properties of melted dark and milk chocolate by means rotational rheometry. The optimum composition of blends of lecithin and PGPR in chocolate systems obviously

depends on processing conditions and the desired product properties and does not allow general recommendations. Emulsifiers blend with a lecithin to PGPR ratio of 30:70.

Schumacher *et al*. (2010) observed a dark chocolate was developed with the addition of 12, 16 or 20 quinoa. The protein concentration of the product increased as the percentage of quinoa increased. The amount of essential amino acid improved in samples containing quinoa. The total polyphenolic content reduced then quinoa was added and the addition of 20 quinoa caused 9 increase in vitamin E, while polyphenols decreased from 23.5 to 18 catechin. Addition or quinoa showed increase in amino acid content of dark chocolate. Sensory analysis revealed that 92 of the sensory panel was very much satisfied with the product quality. A samples were acceptable by the test panel indicating that quinoa can be used in the chocolate adding its potential health benefit to the dark chocolate.

Storage study

Ali *et al*. (2001) studied the effect or 18℃ and 30℃ storage temperatures on texture, polymorphic structure and bloom formation and sensory attributes or dark chocolate stored for 8 weeks. There were significantly retarded changes in filled chocolate stored at 18℃ for 8 weeks. The chocolates were free from bloom during the storage periods. There was an increase in rate of fat migration and rate of change of C36 (carbon number profile % triglyceride) and C50at 30℃ and also a decrease in texture and polymorph structure in coating change to β and β' polymorphs. However the chocolates bloomed in the third week or storage (2 cycles) al that storage temperature. Storage at 18℃ is better than 30℃ and desiccated coconut gives a pleasant flavour to chocolate accepted through the sensory evaluation.

Baylis *et al*. (2004) studied that the survivaI of *Escherichia coli* 0157:H7 and other verocytotoxin-producing *E. coli* (VTEC) in chocolate and other confectionery products. They obtained information on the decline and potential survival of *E. coli*, particuclarly verocytotoxin producing strains, in reduced aw confectionery products - chocolate, biscuit cream and mallow. These products were artificially contaminated with high (4 log10 cfu/ g) and low (2 log10 cfu/g) levels of *E. coli* 0157:H7, 0111: H-026:H11 and their survival as affected by storage temperature (10, 22 and 30℃), was

monitored over 12 months. In chocolate (average aw 0.40), these bacteria were detected for up to 43 days in samples stored at 38℃. At 22℃they survived for upto 90 days and in product stored at 10℃ they could still be detected after 366 days storage.

Salmonella are unable to grow in chocolate because of the low water activity, they are able to survive and persist for long periods of time during storage (Tammingaer *et al*., 1977).

Infective dose of these cells was reported to be much lower than typical levels normally associated with *Salmonella* food poisoning. Similarly, the infective dose of *Escherichia coli* 0157:H7 in hamburgers is also reported to be low, being of the order of less than 100 cells (Wilishaw *et al*., 1994).

Briones *et al*. (2005) studied that migration of fat to the surface of chocolate results in colour changes and development of non-uniform colour patterns. These phenomena are assessed during storage of milk chocolate tablets (cycling temp. between 16℃ and 28℃ for 52 days) by a computer vision system and image analysis.

Nopens *et al*. (2008) developed high resolution quantitative method based on image analysis. The method is able to detect both the evolution or fat bloom in terms of disappearance of gloss and the development of whitish portions at the chocolate surface. The disappearence in fat bloom development rate between samples containing different fat concentration (0. 3 and 69100g) coated on fillings containing different amount of fat 25 and (75g/100g) was distinguish by the application of high resolution quantitative method. In the 25g/100 filling fat, case, blooming occurred at very late stage which was detected due to disappearance of gloss. In 75g/100g filling fat case change in acceptability is due to the development of a whitish surface. High resolution quantitative method based on image analysis can be used in place of procedure.

Chocolate storage is critical to the quality of the final product. Inadequate storage, especially with temperature fluctuations, may lead to a change in crystal structure, which may eventually cause fat bloom. Bloom is the main cause of quality loss in the chocolate industry. The unique chocolate matrix is a mixture of sugar and cocoa solids dispersed in a cocoa butter phase, yet its specific packing structure and

particle interactions make chocolate an even more intriguing and complex substance. Characteristics of chocolate texture are due to both the ratio of solid to liquid fat in a product (solid fat content, SFC) and the crystal state the solid portion is found in, known as the lipid polymorph. A higher SFC affects hardness, melting, and sweetness perception. Chocolate flavor is a combination of volatile compounds for aroma, water-soluble compounds for taste, and physical interactions for mouthfeel. Kinsella, 1989 observed that component volatility largely depends on concentration in the vapor phase, which is influenced by the rate of retention from chocolate and is dependent on temperature, molecular interactions, and partition coefficient of the particular compound. Because chocolate is a continuous lipid phase, most flavor perception occurs due to retronasal action of volatile compounds released during melting. Structural changes of the lipid phase may alter volatile release, thus changing the flavor profile of the chocolate. Most volatile compounds in chocolate are formed via non enzymatic browning during processing. The most potent compounds related to chocolate flavor are the Strecker aldehydes and pyrazines, although the overall flavor profile is a combination of many components, including several sulfur compounds. Chocolate has a shelf life of approximately 12–24 months. As chocolate is stored, structural changes occur. Storage at high temperature with and without fluctuations caused little change in volatile loss or a significant increase in some volatiles as compared to fresh chocolate, possibly due to Maillard reactions. Volatile concentrations were less in chocolate that had transitioned to polymorph VI at 4 weeks and was stored for an additional 4 weeks (8 weeks at 30.5 ± 1.7 °C), signifying that prolonged storage past the polymorphic transition to form VI may exacerbate chocolate volatile loss.

Afoakwa *et al*. (2009) studied fat bloom development and associated changes in microstructure, texture, appearance and melting properties. Dark chocolate varying in particle size were processed and pre-crystallized to under temper regime. A change in texture, surface whiteness, gloss and melting properties evaluated on cooling and after every 24 hour in storage until reaching asymptotic value and bloom was induced by storing product under ambient condition (18-20°C, RH 50). Stereoscopic binocular microscopy was used to characterize the microstructure of product during blooming. Until reaching asymptotic levels, measurement on texture and surface whiteness showed initial rapid increases with consequential reduction in gloss within the first 96

hours followed by gradually decreasing gradient. After tempering micrographs showed similar surface crystalline network structure and inter particle interactions among products from different particle size (PS) and bloom initiation occurred within 24 hour in storage resulting in appearance of both liquid and unstable fat on surface of products. The unstable fat then re-crystallized during storage into more stable polymorphs and crystal growth was promoted by Oswald ripening with the appearance of white crystalline structure which spread gradually throughout the chocolate mass after 96 hour. Attributed mainly to hydrodynamic force by capillary action the product containing the largest (PS 50 μmeter) showed the fastest fat bloom rate with the smallest PS (18μm) the least.

The effect of active and modified packaging as well as packaging material oxygen permeability and on quality retention of dark chocolate with hazelnuts studied by Mexis *et al.* (2010). Dark chocolate was packaged in: a) polyethylene terephthalate /low density polyethylene (PET IILDPL) and b) polyethylene terephthalate coated with Si0x low density polyethylene (PET-Si0xt//LDPE). Samples were packaged either under vacuum or N2 or with an oxygen absorber and stored in the dark at 20℃ for a period of 12months. Commercial control samples for comparison purposes consisted of chocolate packaged in aluminum foil in air while "model" control samples used for sensory evaluation consisted of chocolate packaged in glass jars and stored at - 18℃. Quality parameters monitored were: peroxide value, hexanal content, colour, fatty acid composition and volatile compounds The sensory attributes - colour, texture, odour and taste were evaluated. PV ranged between 0.80 for fresh dark chocolate with hazelnuts and 6.51 meq O2/kg chocolate fat for commercially packaged samples after 12 months or storage. Respective values for hexanal were 0.53 and 7.56 mg/kg. Saturated fatty acids (SFA) increased with a parallel decrease in monounsaturated fatty acids (MFA) and polyunsaturated fatty acids (PUFA) after 12 months of storage mainly in least protected samples (Commercial package). Likewise, after 12 months of storage an increase in concentration or aldehydes, ketones, alcohols and alkanes ($p>0.05$) with a parallel decrease in pyrazines were observed especially in case of least protected products after 6 and 12 months of storage.

Jassun *et al*. (2011) studied che capacity to detect low levels of healthy and sub- lethally injured *Salmonella enterica* cells in chocolate by two alternative rapid

detection methods-IQ-Check *Salmonella* II real-timeime PCR (Bio-Rad) and VIDAS Easy SLM (BioM6ricux) as assessed and compared with ISO 6579:2005. Chocolate, a low moisture food known to support the survival or *Salmonella* was challenged as food matrix. Buffered peptone water (BPW) did not support the recovery of low levels of sub-lethally injured *Salmonella enterica* independent of the detection method while BPW supplemented with milk powder enabled detection by the three examined methods. However, inhibition of real time PCR was observed since for one out of three repetitions of chocolate inoculated with a low number of sub-lethally injured *Salmonella enterica* cells, no PCR signal was obtained. He told that attention should be paid to the enrichment step to avoid false negative results due to the presence of sub-lethally injured *Salmonella* cells in chocolate.

An appropriate sample preparation (such as enrichment media and conditions for incubation) remains the key factor for reliable detection including sub-lethally injured cells and should be evaluated Salmonella can not grow in chocolate but has the ability to survive for a prolonged period (Daoust *et at*., 1975).

A significant effect of the storage temperature on the intensity of changes in chocolates was reported by Machalkova *et al*. (2014). There was a product-specific intensity of positive effects of retempering observed in the products.

A critical step is product development is determining the safety and shelf life of the product by measuring the moisture content and water activity. Moisture content measures the amount of free water within a system and indicates the perishability of the food (Ward, 2007).

Health benefits

Steinberg *et al*. (2003) studied on cocoa and chocolate flavonoids. Flavonoids are round in cocoa and chocolate. According to recent reports the main flavonoids found in cocoa, flavan3-ols and their oligomeric derivatives, procyanidin. have a variety of beneficial actions, including antioxidant protection and modulation of vascular homeostasis. Other constituents in cocoa and chocolate that may also influence cardiovascular health are briefly reviewed. One third of the lipid in cocoa butter is composed or the stearic acid, which exert a neutral Cholesterol emic response

I in humans. However the lipid content of chocolate is relatively high. Intake of trace mineral, which is necessary for optimum funtioning of all biologic systems and for vascular tone contributed by cocoa and chocolate. Thus multiple components in chocolate, particularly flavonoids can contribute to the complex interplay of nutrition and health. This study helps to encourage individuals to consume a wide range of phomchemical-rich foods which can include chocolate in moderate amounts also recommended by health professionals

Carresecchi *et al.* (2005) studied that the effects of cocoa powder and extracts with different amounts of flavanols and related procyanidin oligomers investigated on the growth or Caco-2 cells. Treatment of the cells with 50 ug/ml of procyanidin enriched (PE) extracts caused a 70% growth inhibition with a blockade of the cell cycle at the G2IId phase. PE extracts caused a significant decrease of ornithine decarboxylase and S-adenosylmethionine decarboxylase activities, two key enzymes of polyamine biosyruhesis. This led to a decrease in the intracellular pool of the polyamines These observations indicate that polyamine metabolism might be an important target in the anti-proliferative effects of cocoa polyphenols.

Mursu *et al.* (2005) reported that cocoa powder is rich in polyphenols and hence contribute to the reduction of lipid peroxidation. Cocoa polyphenols increased the concentration of HDL cholesterol, whereas chocolate fatty acids may modify the fatty acid composition of LDL and make it more resistant to oxidative damage.

Grasssi *et al*. (2010) reported that cocoa flavonoids are able to reduce cardiovascular risk by improving endothelial function and decreasing blood pressure (BP). Interest in the biological activities or cocoa is daily increasing. A recent meta-analysis showed that flavanol rich cocoa administration decreased mean systolic (-4.5 mm Hg; $p<0.001$) and diastolic (mm Hg; $p<0.00l$) BP The risk of cardiovascular diseases has been decreased by 3.mm Hg systolic BP. Cocoa consumption could play a pivotal role in human health.

Tarin *et al*. (2011) reported that chocolate has emerged as a possible modulator of cardiovascular risk. Cocoa as the natural source, contains flavanols, a subclass of flavonoids. Oxidative stress, inflammation and endothelial function defined three biological mechanisms that had shown sensitivity to chocolate.

Moreover, the consumption of chocolate has been involved in the protective modulation of blood pressure, the lipid profile, the activation of platelets and the sensitivity to insulin. Dark chocolate seems more protective than milk or white chocolate.

Khan *et al*. (2012) reported suggest that regular consumption of cocoa containing products may confer cardiovascular protection reducing the risk of coronary heart disease (CHD).

Martin *et al*. (2013) studied that Cocoa and its main polyphenols interfere in the initiation, promotion and progression of cancer and prevent cancer. Cocoa flavonoids influence several important biological functions in vitro and in vivo by their free radical scavenging ability or through the regulation or signal transduction pathways to stimulate apoptosis and to inhibit angiogenesis and metastasis.

Vazquez-Agell *et al*. (2013) reported that the health effects of cocoa polyphenols may be due to their antioxidant and anti inflammatory actions. Although the exact mechanisms are unknown and depend on the matrix in which cocoa-polyphenols are delivered. Nuclear factor □B (NF-H□B) is a key molecule in the pathophysiology of atherosclerosis involved in the regulation of adhesion molecules (AM) and cytokine expression and its activation is the first step in triggering the inflammatory process

Miller *et al*. (2006) Cocoa and chocolate products have been used as medicinal remedies, symbols of luxury and palatable sweets for hundreds of years. Cocoa and some chocolate products have among the highest concentrations of polyphenols (flavonoids, catechin and epicatechin; 3.3-60.2 mg/g of GaIlic acid equivalent) compared to other food sources of polyphenolic compounds (i.e. fruits, vegetables. Lea, and red wine; 0.5-7.I mg/g or Gallic acid equivalent). Due to the content of these polyphenol antioxidants in cocoa and chocolate products, they may provide protection against oxidative stress.

Habitual chocolate intake was related to cognitive performance, measured with an extensive battery of neuropsychological tests (Crichton *et al*., 2016). More frequent chocolate consumption was signi**fi**cantly associated with better performance

on the Global Composite score, Visual-Spatial Memory and Organization, Working Memory, Scanning and Tracking, Abstract Reasoning, and the Mini-Mental State Examination. With the exception of Working Memory, these relations were not attenuated with statistical control for cardiovascular, lifestyle and dietary factors. The ability of flavonoid-rich foods to improve cognitive function has been demonstrated in both epidemiological studies (Letenneur *et al*., 2007 and Nurk *et al*., 2009) and clinical trials (Macready *et al*., 2009).

Kruger *et al*. (1999) reported that fine crystalline sucrose is utilized at up to 50 % in chocolate confectionery. Jefffery (1993) studied that epending on the type of chocolate, sucrose constitutes more than 40-50 of'solids dispersed in fat and thus. Its functional properties including sweetness, stability, particle size distribution, mouthfeel (texture). and its impact on rheological properties of the product are important for chocolate products.(Moskowitz, 2001, Olinger *et al*., (2001) and Strater, 2001).

Chocolate contains antioxidants that are associated with positive health benefits (Patel *et al*., 2008). In recent years, several studies described chocolate as a functional food (Dillinger and Barriga, 2000; Kelly, 2005, Schinella *et al*., 2010 and Visioli *et al*., 2009). Chocolate is a fascinating food; its indulgent qualities, concentrated energy and unique nutritional components distinguish chocolate from other foods. Numerous research studies report that polyphenols in cocoa are biologically active and have the potential to release stress as reported by Liu (2011).

Oatmeal has been approved as a heart healthy food by the US Food and Drug Administration (FDA) because of its bulky fiber content and low saturated fat content. One cup of oats contains 3.98 g of dietary fiber (15.92% DV), 5.94 g of proteins (11.88% DV) and 0.73 g of saturated fats (3.65% DV). Oats are also a very good source of manganese, selenium, magnesium, zinc and phosphorous. These are essential minerals that are needed for the body to function properly. Oats are also considered to be a part of the 18 gluten-free diet, because only a small amount of gluten may be present and is tolerable by many adults and children with celiac disease (Lehtinen *et al*., 2009).

Oatmeal, being a whole grain cereal, contains many of the nutrients that are beneficial to the heart. A 19.6 yearlong study (Djousse and Gaziano, 2007) determined that the consumption of whole grain cereal lowers the risk of heart failure. Another study (Bazzano *et al*., 2003) showed that eating 21 g of fiber per day lowered the risk coronary heart disease and cardiovascular disease by 12% and 11%, respectively, when compared to consuming only 5 g of fiber per day. Oats contain a specialized type of fiber called beta-glucan that assists in a heart healthy claim. Fiber also lowers cholesterol levels by interacting and removing LDL cholesterol from the digestive system (Othman, 2011).

Another compound linked with cholesterol is avenanthramide. This unique antioxidant compound is only found in oats and protects cholesterol by preventing free radical damage to LDL cholesterol (Chen *et al*., 2004).Oats contain a significant amount of other compounds with antioxidant activity such as vitamin E. Antioxidants protect the body from free radicals that are known to cause several types of diseases, cancers and aging (Ryan *et al*., 2011). Feeding curry leaves to rates produced hypoglycemia by increasing the hepatic glycogenesis as evident by increased activity of glycogen synthetase. A decrease in glycogenolysis and gluconeogenesis is reported and was evident form decreased activity of glycogen phosphorylase and gluconeogenicenzymes (Manfred *et al*., 1985).

A significant reduction in fasting blood sugar and postprandial blood sugar was observed by feeding (12gm) leaves powder to Non-Insulin Dependent Diabetes Mellitus patients (NIDDM) (Khan *et al*., 1985 and Felicia *et al*., 1993). The excellent pharmacological potential of mahanimbine from curry leaves to prevent obesity was reported by Birari *et al*. (2010)

The protective nature of *M. koenigii* leaves extract was studied by Gupta and Singh, 2007. The effect attributed to the combined effect of carbazole alkaloids – Mahanimbine, Girinimbine, Isomahanimbine, murrayazoline, Murrayazolidine, Mahanine and ascorbic acid, α-tocopherol and mineral (Zn, Cu,Fe) contents of *M.koenigii*leaves extract. This study proved *M. Koenigii* a promising and a rich source of free radical quenchers, which have been mediated through hepatocyte membrane stabilizing activity alongwith the reduction of fat metabolism. The normal

morphology of cell was maintained after ethanolic challenge when aqueous extract containing tannins and carbazole alkaloids of *M. koenigii* was given. *M. koenigii* leaves possess good antioxidant activity in vitro and are able to protect against radiation-induced depletion in cellular antioxidants as reported by Deepa and Devi (2009).

M. koenigii, has vast number of therapeutic applications such as in bronchial disorders, piles, vomiting, skin diseases etc. The medicinal utilities have been described especially for leaf, stem, bark and oil. The leaves of *M. koengii* are used as tonic, stomachic, carminative, internally in dysentery, antiemetic, antihelminthic, Following various claims, efforts have been made by the researchers to verify the efficacy of the plant through scientific biological screening and analgesic. (Dhongade *et al*., 2013)

Response surface Methodology RSM

Minitab 17 is the particular software used here. RSM is reported to be an effective tool for optimizing a process when the independent variables have joint effect on the derived response (Hunter, 1959). Several workers have used it for optimization of cake formulation and reconstitution studies (Donelson and Wilson, 1960, MacDonald and Bly, 1968 and Kissel and Marshall, 1967). For storage studies under constant temperatures, kinetic constants are determined for the appropriate model and then used to stimulate storage behaviour (Mizrahi and Karel, 1978). The RSM can be successfully used to optimize a process (Mudahar *et al*., 1989 and Genser *et al*., 1987). Henika (1972) described response surface analysis as a useful statistical tool for analyzing experimental data to optimize the physical properties of the food products using different levels of ingredients. Since then, RSM has been used in several areas like studies of protein denaturation (Neilson *et al*., 1997), bacterial growth (Schroder and Busta, 1973) etc. During storage the quality of the product gets deteriorated due to chemical and enzymatic reactions, which in turn changes the properties of the product and thus affects its overall acceptability. The development of food products and or process is a complex expensive and risky multistage process. and special requirements should he considered in this process such as consumer demands, price, operational conditions and legislation background. To develop or to optimize processes, many companies come up statistical approaches such as response

surface methodology (RSM). In food and chemical companies RSM has important applications in the design analysis and optimization of existing products and unit operations and its use decreases the volume of experiments, reagents, time financial input energy) along others (Montgomery, 2009). Myers *et al*. (2004) also reported significant application of RSM in product optimisation.

Chapter-3

MATERIALS AND METHODS

The present study entitled, **"Process Optimization of Oat Based Functional Chocolate and its Shelf Life Evaluation"** was carried out at Centre of Food Science and Technology, Institute of Agriculture Sciences, Banaras Hindu University, Varanasi, India. During this study, the work done was planned to optimize the combination of ingredients in the mixture to be used in preparation of dark chocolate enriched with oats and curry leaves and their interaction influencing the sensory quality and properties of product. Also, the study was done to analyze the physico-chemical properties and the storage stability of optimized product was also carried out based on sensory parameters like appearance, colour, flavour and texture of soup and chemical parameters like acidity, protein, fat, phenolic content etc were also analysed. Different materials were used and followed during the period of study and are described in a sequence below.

3.1 Sources of raw materials

Dark Chocolate : Amul Dark Chocolate by AMUL INDIA

Oats : Kellog's rolled oats by KELLOG INDIA PVT. LTD

Curry Leaves : Procured from local market of Varanasi

Sugar : Procured from local market of Varanasi

3.2 Materials and equipments used in the preparation and analysis of fortified dark chocolate

3.2.1 Materials used for making Functional Chocolate are as follows:

1. 1.Amul Dark Chocolate
2. 2.Oats (roasted and powdered)
3. 3.Curry leaves (dried and powdered)
4. Sugar (powdered)
5. Chocolate wrapping paper

6. Stirrer
7. Knife
8. Glass bowl
9. Containers for double distillation
10. Mould

3.2.2 Equipment

1. 1.Weighing balance
2. Refrigerator
3. Mixer
4. 4 Desiccators
5. TA.XT plus profile analyzer
6. SOCS PLUS SCS4
7. Kjeldahl Apparatus: Kel Plus - Elite EX (VA)
8. Hot Air Oven
9. Blender
10. Muffle furnace

Analytical grade chemicals and reagents were used for the analysis.

3.3 Plan of work

Keeping in the view the objective of the investigation, the plan of work is discussed here as under.

Optimization

The processing parameters or factors were optimized with respect to the responses viz. body and texture, colour and appearance, flavour, overall acceptability, hardness and of the biscuits. Numerical optimization technique of the Minitab17 software was used for simultaneous optimization of the multiple responses. The desired goals for each processing parameter or factor and response and response were chosen. The goals may be applicable to either processing parameters or factors and responses. The possible goals or constraints are: Maximize, Minimize, Target, and within range and set to an exact value (for processing parameters or factors only). In order to search a solution, the goals were combined into an overall composite function, called the desirability function; the maximum value of the function is unity. The response surface help to understand the effect of varying the processing

parameters or factors upon the response, i.e. in which direction the response is increasing or decreasing. Response surface were generated with the help of statistical package (Minitab17).

Statistical optimization

Response surface methodology which involves designing of experiments, selection of levels of variables in experiments runs, fitting mathematical models and finally selecting levels of variables by optimizing the response was used in the study (Khuri et al., 1987). A central composite rotatable design (CCRD) (Lorezen et al., 1993) was used to design the experiments comprising three independent processing parameters or factors. Twenty experiments were conducted for the present research work. There were six experiments at centre point to calculate the repeatability of the method (Montgomery, 2001). The experiments were conducted in randomized order to minimize the effect of unexpected variability in the observed responses because of extraneous factors. The experimental design and the codes for the processing parameters or factors are reported in table 3.1. Response surface analysis required coding of the values of the processing parameters of factors are oat, curry leaves and Dark chocolate. The independent variables, the lower and upper limit has been shown below

Table 3.l Independent Variables used for optimization

INDEPENDENT VARIABLES	SAMPLE CODE	CODED VARIABLES		
		-1	0	1
Dark Chocolate	A	30	40	50
Oats Powder	B	14	19.5	25
Curry leaves Powder	C	01	2.5	04

Statistical Analysis of Data

The experimental data obtained was analyzed with the help of Minitab17 software. After that each individual experiment, responses were analyzed to assess the

effect of independent parameters or factors on them. The second order polynomial equation of the following form was fitted to the responses:

$Yk = \beta o + \Sigma \beta i\ Xi + \Sigma \beta ii + X^2 + \Sigma \beta ij\ XiXj$

Where

Y= Responses

βo, βi, βii, and βij = constant, linear, quadratic, and cross product regression coefficient

X= are the actual value of the independent variables.

As per response of' sensory parameters body and texture, color and appearance, flavour, overall acceptability parameters of hardness, chewiness and stringiness were selected on which the effect of the three individual ingredients has to be evaluated. A combination of' 20 number of experiment were generated (Table 3.2) in Minitab l7 using Response surface methodology during the investigation.

Table 3.2 Experimental design for analysis and optimization of the product

RUN ORDER	(A) DARK CHOCOLATE	(B) POWDERED OATS	(C) POWDERED CURRY LEAVES
1	40.0000	19.5000	3.00000
2	40.0000	19.5000	3.00000
3	40.0000	19.5000	3.00000
4	40.0000	19.5000	1.31821
5	56.8179	19.5000	3.00000
6	40.0000	19.5000	3.00000
7	30.0000	14.0000	2.00000
8	40.0000	19.5000	4.68179
9	40.0000	10.2501	3.00000
10	40.0000	28.7499	3.00000
11	40.0000	19.5000	3.00000
12	23.1821	19.5000	3.00000
13	50.0000	14.0000	4.00000
14	40.0000	19.5000	3.00000
15	30.0000	25.0000	2.00000
16	50.0000	25.0000	4.00000
17	30.0000	25.0000	4.00000
18	30.0000	14.0000	4.00000
19	50.0000	14.0000	2.00000
20	50.0000	25.0000	2.00000

Fig. 3.1 Fresh curry leaves

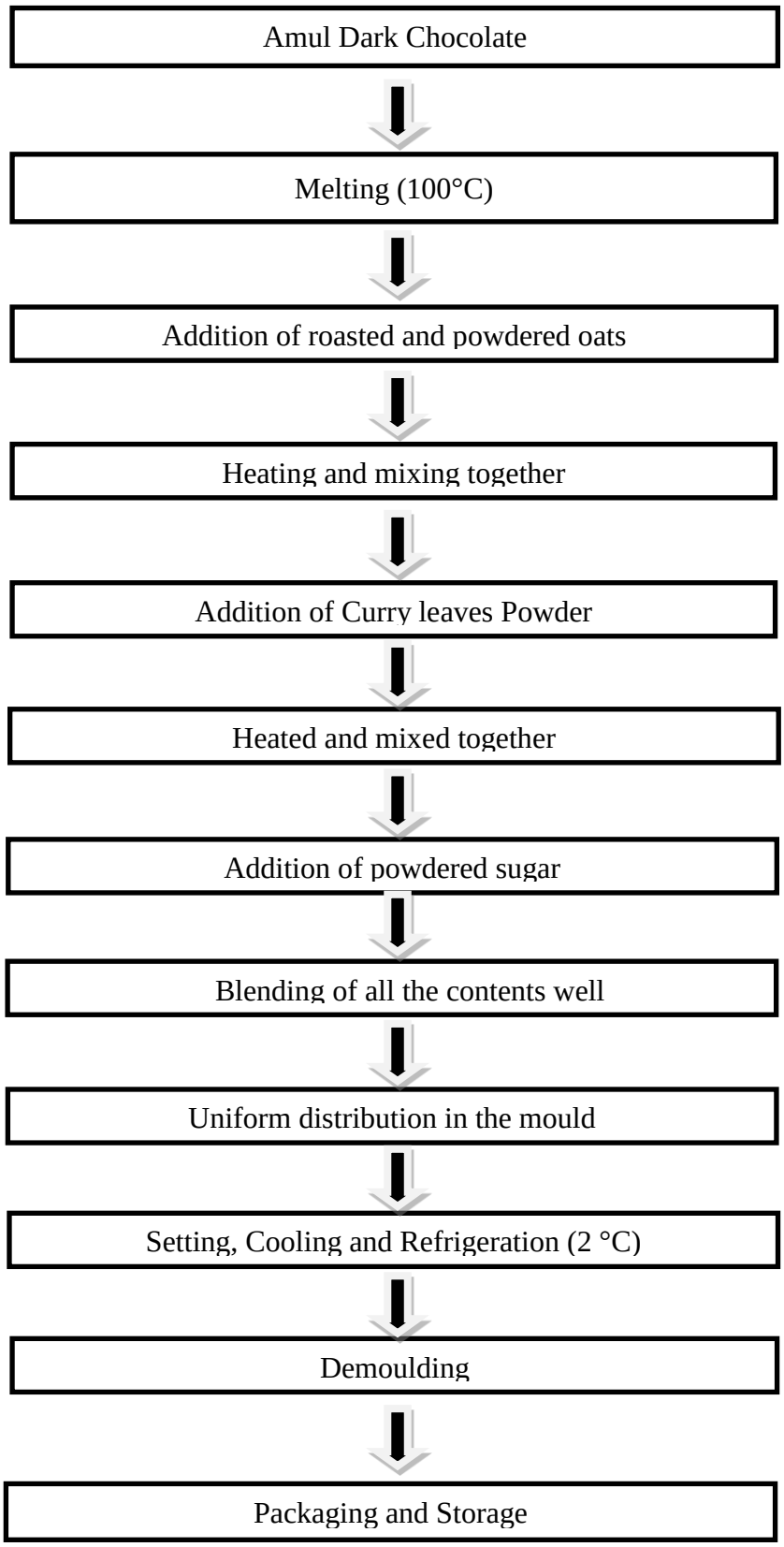
Fig. 3.2 Curry leaves powder

Fig. 3.3 Oats

Fig. 3.4 Roasted oat powder

Fig. 3.5 Product

Fig.3.6 Method of preparation of functional dark chocolate



3.3.1 Method for preparation of chocolate

The following ingredients and quality were used for preparation of chocolate.

Table 3.3 Ingredients of chocolate

INGREDIENTS	AMOUNT (g)
Dark chocolate	65.4108937
Oat powder	32.90899
Curry leaves powder	1.53014177
Sugar	8

For preparation of chocolate, desired amount of dark chocolate was heated at 100℃ for its melting and oats and curry leaves powder were mixed in it homogenously. Sugar powder was later added to it. The contents were mixed well to obtain a homogenous and smooth paste. Then, immediately the mixture was poured it was tempered manually on a cool surface onto 1 the mould and was refrigerated at 2℃for proper crystallization of chocolate. The prepared product was then packaged properly in Chocolate wrapping paper and stored at 4°C temperature for further analysis.

3.4 Sensory evaluation

A sensory score card suggested by Williams (2001) (Scale: 1-9; 1 -most disliked and 9-most liked) with little modification was adapted to analyze the sensory characteristics of the developed chocolates. Sensory evaluation of functional dark chocolate was performed by a panel of 5 semi trained judges from the Centre of Food Science and Technology at Banaras Hindu University, Varanasi, India.

3.5 Physical characteristics of fortified dark chocolate

3.5.1 Texture analyses (TA)

Textural parameters of product like hardness, stickiness and stringiness were analyzed using Texture Analyzer (TA.XT plus Texture profile Analyzer Stable Micro systems, UK).

Table 3.4 Texture analyses setting for Texture profile analysis of Dark Chocolate

TA Settings: Mode	Measure Force in compression
Option	Return to Start
Pre-test Speed:	1.0
Post-Test Speed:	2.0
Distance:	2mm
Trigger l:orce:	5g
Tare Mode:	Auto
Data Acquisition Rate:	50pps
Accessory : 3 Point Bending Rig (hdpbs) Stage	

3.6 Data analysis and optimization method

The statistical analysis was done by Response Surface commercial statistical software package Minitab 17 for fortified dark chocolate.

3.7 Proximate analysis of Optimized product

3.7.1 Reducing Sugar

Principle

The DNS (Dinitro salicylic acid) method for estimating the ion concentration of reduing sugar in a sample was originally reported by G. Miller (1959). Reducing sugar has property to reduce the reagent. A reducing sugar in one that in a basic solution forms an aldehyde or ketone. The aldehyde group of glucose convert 3,5-dinitro salicylic acid (DNS) to 3-amino-5 nitro salicylic acid. Water is used up as a reactant and oxygen gas is released duiring the reaction.

The formation of 3-amino-5nitro salicylic acid results in a change in the amount of light absorbed at wavelength 540nm. The absorbance measured using a spectrophotometer is directly proportional to the amount of reducing sugar.

Material

Sodium Potassium Tartrate: Dissolve 45gm of' NaOH tartrate in 75ml of H2O (1)

3,5-DNS solution

Dissolve 1.5gm of DNS reagent in 30ml of 2M NaOH (2)

2 Molar NaOH

80gm of NaOH dissolved in l litre of H2O

DNS reagent

Prepare fresh by mixing the reagent (l) and (2) make the volume to 150 ml with water using standard sugar sodium.

Standard Sugar sodium.

a) Stock the standard sugar sodium 250mg of glucose in water and make up the volume to 100ml.
b) Working with standard sodium: take 10ml from this stock solution and make the volume to 100ml.

Procedure

1. Take clean and dry test tube
2. Pipette out the standard solution in the range of to 2ml (0. 0.5. 1.5. 2.0)
3. Make the final volume in all the tubes to 2ml with distilled water
4. Add 1ml DNS reagent to all the test tube and mix will and capped it (To avoid the loss of liquid to due to evaporation).
5. Keep the test tube in boiling water bath for 10 minute (temperature 100°C)
6. Take the tube and cool to room temperature read extinction al 540nm against the blank.
7. Prepare the standard curve of the sugar provided and use them to estimate the concentration of unknown sample provided.

Procure the result as: The 100ml of unknown solution contain __ mg of glucose.

3.7.2 Non-Reducing Sugar

Non- Reducing Sugar = Total Sugar - Reducing Sugar

3.7.3 Antioxidant analysis

3.7.3.1 Total Phenolic content

Method: Folin-Ciocalteau

The total phenolic content was determined by (Singleton and Rossi, 1965; Kaur and Kapoor, 2002). Samples (2g) were homogenised in 80% ethanol at room temperature and centrifuged in cold at 10,000 rpm for 15 min and the supernatant was extracted. The residue obtained was re-extracted twice and the supernatents were collected, poured into petridishes and evaporated to dry at room temperature. Residue was dissolved in 5 ml of water. 100µml of this extract was diluted to 3 ml of water and 0.02ml of Folin-Ciocalteau reagent was added. After 3min, 2ml of 20% sodium carbonate was added and the contents were mixed thoroughly. A colour developed and the absorbance was measured at 750nm using UV-spectrophotometer using Gallic acid as a standard. The results were expressed as mg Gallic acid/100g fresh material

3.7.3.2 DPPH radical scavenging assay:

DPPH radial scavenging assay was based on the modified method of Brand-Williams *et al*., 1995 and Michalska *et al*., (2007). In this assay antioxidants present in the sample reduce the DPPH radicals. For preparing the extract, sample (1ml/10ml) for cenrifuged at 10,000rpm for 15 min. The supenatant collected was used in the assay.

The DPPH radical solution

It was prepared by dissolving 10mg of DPPH in 25ml of 80% ethanol.

Sample preparation

The bank sample was prepared by adding 0.2ml water to 1ml DPPH solution. Then, after 30 min incubation,dilution was performed in ethanol blank and sample solution.

3.7.4 Protein Estimation

The protein content in fortified chocolate was estimated by adopting the following protocol (AOAC,2000).

Digestion

The system was switched ON and the digestion unit was pre-heated upto 350°C. 0.2g sample was weighed (w) using the filter paper. The samples were prepared in triplicates. The samples were taken in a 250ml Macro DTL tube. Then 10ml of conc. Sulphuric acid was added, followed by the addition of 4g of catalyst mixture (Potassium and copper sulphate in 5:1). The sample tube was loaded in the digestion unit with manifold % KEL FLOW setup. Tap water was connected with maximum pressure for KEL FLOW. The temperature was increased to 42°C. Digestion was carried out till the clear green colour appeared. 20 min were assigned for digestion and after that the digestion mixture was cooled on the cooling rack for 20 min approx.

Distillation

The system was switched ON and the solutions viz. 4% boric acid, 40% alkali and 0.1N HCl was prepared and kept. The alkali, boric acid and potassium permanganate were loaded to the system through silicon hosed provided at the back of the equipment while waiting for the READY signal. In a 250 ml conical flask, 25 ml boric acid was taken with the indicator and placed at the receiver end. The sample was diluted with distilled water (10-20ml). Then, the sample tube was loaded in the sample side. Before starting the sample testing, water was allowed to flow through the system for cooling purpose (Check the INLET and OUTLET). The sample testing was started after the READY signal appeared. 40 ml of the 40% alkali was added to the above solution (until dark brown colour appears). The process was started and liquid ammonia was collected into beaker having boric acid where its colour changed according to the indicator used. After completion of the process the conical flask was removed from the receiver end and then titrated. The DTL tube from the sample side was then removed.

Titration

0.1N HCI was taken in the burette and titrated against the blank and then against sample. The burette values were noted down.

Calculation

$$N\ (Nitrogen)\ \% = \frac{14.01 \text{ x } 0.1\text{N x } (\text{TV} - \text{BV}) \text{ x}100}{\text{Weight of sample x } 1000}$$

Protein% = %Nx6.25 (for Food Samples)

Where,

TV = Titre Value, BV = Blank Value and W = Sample Weight.

3.7.5 Estimation of Fat

The fat content in fortified dark chocolate was estimated by the following protocol (AOAC, 2000).

Procedure

5g homogenized sample was taken in a thimble and it was placed in previously weighed Soxhlet beaker. The beakers were then placed in the extractor (SOCS PLUS). Then the extractor was filled with petroleum ether and their tops were covered with cotton plugs. The Soxhlet apparatus was then switched on with a set temperature of 70°C for 1.5h. After completion of extraction, the temperature was increased upto 130°C for 10 min for the complete removal of moisture. The beakers were removed from SOCS PLUS apparatus and cooled in desiccators. The cooled beakers were then weighed

$$Fat\% = \frac{\text{w2} - \text{w1}}{S} \text{x } 100$$

Where,

Weight of residue = Weight of beaker after drying (W2) - weight of empty beaker (W1).

S = Weight of Sample.

3.7.6 Ash Estimation

The Ash content in the fortified dark chocolate was estimated by following the protocol (AOAC, 2000).

Procedure

5g of completely homogenized sample was taken accurately in moisture free silica crucible. The crucibles were then placed on hot plate at 130°C till smoke disappeared. After this, the crucibles were placed in muffle furnace at 550°C (6 h). Weights of the cooled crucibles were noted down.

Calculation

$$\text{Ash\%} = \frac{w2-w1}{S} \times 100$$

Where, W1 is the weight of Silica dish+Ashand S is the weight of sample.

3.7.7 Moisture content

Moisture content in the fortified dark chocolate sample was estimated by following protocol AOAC, 2000). In washed pre-heated, cooled and weighed empty dishe,. 5g of samples was weighed in triplicates. The dishes were placed in pre-heated, hot air oven at 450±5°C for 24 h. After drying, the dishes were cooled in the desiccators and weighed.

Calculation

$$\text{\%Moisture content} = \frac{w2-w1}{\text{Weight of Sample}} \times 100$$

Where, W2 = weight after drying and W1= initial weight of sample + weight of petridish.

3.8 Determination of microbial population

Preparation of the samples (serial dilution) I ml of sample was taken and transferred to the test tube with 9ml of normal saline solution (0.9%NaCl). The samples were serially diluted up to 10^{-2} dilution.

3.8.1 Simple plate count

Total Plate Count (TPC) was used for determination of bacterial count.

Method

Sterlisation: The prepared media was heated for 15 min in an autoclave maintained at 15psi for sterilization at 121°C. All glasswares and necessary items were properly autoclaved to avoid contamination.

Pouring: It was done in the laminar air flow chamber. The flame was highly lighted and petri dishes were slightly opened near the flame and the media was poured in the petri-dishes and kept for solidification.

Inoculation of sample: Inoculation was done aseptically in laminar air flow chamber by taking 0.1g of the sample suspended in saline solution from 10^{-2} transferred to petri-dish with a label 10^{-2} of nutrient agar media. Similarly, all the samples suspended in saline solution and transferred into the respective petri-dishes of nutrient agar media. Duplicate sample were taken for each dilution and a control of nutrient agar media was also kept without inoculatian. The inoculated petri-dishes were incubated in incubator for 24h at 37±1°C temperature. Total plate count was noted after 24h.

TPC (CFU/ml) = No. of colonies/dilution factor x0.1

Where, CFU= Colony Forming Unit

Amount plated=0.1g

3.8.2 Coliform count

Violet red bile agar was used for this purpose.

Method

In laminar air flow, the media was poured in sterile petridishes in hot condition and kept till it solidified. The plates were marked according to the samples

in duplicates of each. l00mg of sample was weighed in the sterilized beaker and it was added to the first dilution tube and mixed thoroughly and was then serial diluted till 8th dilution was achieved. The 8th diluted sample was then plated on solidified agar using spread plate techniques. The plates were then incubated at 37°C for 48h in inverted position. The colonies were then counted.

3.8.3 Yeast and mould

PDA (Potato Dextrose Agar) was used to determine the yeast and mould in the product.

Method

Sterilization:

The prepared media was heated for 15 min an autoclave maintained at 15 psi for sterilization at 121°C. All glasswares and necessary item were properly autoclaved to avoid contamination.

Pouring:

Pouring was done in the laminar-air flow chamber. The flame was lighted and petridishes were slightly opened near the flame and the media was poured in the petridishes and kept for solidification.

Inoculation of sample:

It was done aseptically in laminar air flow chamber by taking O.l g of the sample suspended in saline solution from 10^{-2} and transferred to petridishes with label 10^{-2} of nutrient agar media. Similarly, all the samples suspended in saline solution were transferred to the respective petridishes of nutrient agar media. Duplicate samples were taken for each dilution. A control of nutrient agar media was also kept without inoculation. The inoculated petridishes were incubated in incubator for 72h at 25°C temperature. Colonies were counted after 72 h.

TPC (CFU/ml) = No. of colonies/dilution factor x0.l

Where, CFU= Colony Forming l unit

Amount plated=0.1g

3.9 Storage study of optimized chocolate

Shelf life study was done with the optimized chocolate samples. Chocolate sample were wrapped in Chocolate wrapping paper and then packed in polythene pouches. Properly packaged chocolate samples were then stored at two different temperatures. The two different temperatures were taken as 4°C and 25°C. Normally chocolate is stored in refrigerated condition because its melting point is low. However, the storage temperature was also selected as 25 to assess the quality of shelf life. Storage stability was tested based on sensory parameter like appearance, color, body andtexture, flavor and mouth feel and overall acceptability.

Chapter-4

RESULTS AND DISCUSSION

On the basis of preliminary experiment and literature available, the level of all independent variables such as dark chocolate, roasted oat powder was decided to analyse the effect on response. Experiment was designed using RSM and data are presented. Each of the individual response was analysed to measure its variability with independent process variables.

4.1 Sensory Evaluation

Body and texture

The texture analysis was performed using the texture analyser. The texture was found greatly affected by the presence of higher amounts of oat powder.

Color and Appearance

The color and appearance of the products during sensory evaluation was significantly affected by amount of dark chocolate and oats.

Flavour and taste

The taste of product during sensory evaluation was significantly affected by curry leaves powder. Chocolate, however, due to being the major component, imparts the characteristic dark chocolate essence but is affected by oat powder.

Overall Acceptability

The sensory evaluation of the the product at the two different temperatures 4 and 25°C was done and overall accepatabilty was calculated. The chocolates samples stored at the lower temperature of 4°C showed appreciable values.

4.2 Texture Analysis

Hardness

The hardness of the different samples was noted and the influence caused due to higher % of oats was considerably noted.

Chewiness

The product developed chewiness due to the presence of oat fibers. The dark chocolate and Curry leaves didn't contribute to chewiness significantly.

Stringiness

The Stringiness of product during Texture analysis was significantly affected by dark chocolate and oats.

4.3. Optimisation of the product

Table 4.1 RSM for optimization of product

DC	Oats	CL	Hardness	Chewiness	Springiness	BandT	CandA	Flavour	OAA
40	19.5	3	1852.062	25586.63	0.656	7	7	7	7
40	19.5	3	1852.062	25586.63	0.656	7	7	7	7
40	19.5	3	1852.062	25586.63	0.656	7	7	7	7
40	19.5	1.31821	1675.125	55688.13	0.042	9	9	9	9
56.8179	19.5	3	2030.131	43795.74	0.683	8	9	7	8
40	19.5	3	1852.062	25586.63	0.656	7	7	7	7
30	14	2	2261.105	21743.53	0.556	7	8	8	8
40	19.5	4.68179	1912.183	33069.55	0.978	7	6	5	6
40	10.2501	3	1784.008	7387.137	1.112	8	8	7	8
40	28.4799	3	2451.019	43974.64	0.635	5	5	7	6
40	19.5	3	1852.062	25586.63	0.656	7	7	7	7
23.1821	19.5	3	2689.015	49008.45	0.467	6	5	7	6
50	14	4	1545.121	25822.13	0.629	9	8	6	8
40	19.5	3	1852.062	25586.63	0.656	7	7	7	7
30	25	2	2513.148	25586.63	0.656	7	7	9	8
50	25	4	1645.013	1458.33	0.383	8	8	6	7
30	25	4	2451.061	55870.2	0.045	7	7	6	7
30	14	4	1546.101	7237.599	0.482	8	8	6	7
50	14	2	1503.051	36442.19	0.973	9	9	9	9
50	25	2	1521.085	16530.82	0.398	8	9	8	8

Optimisation Analysis

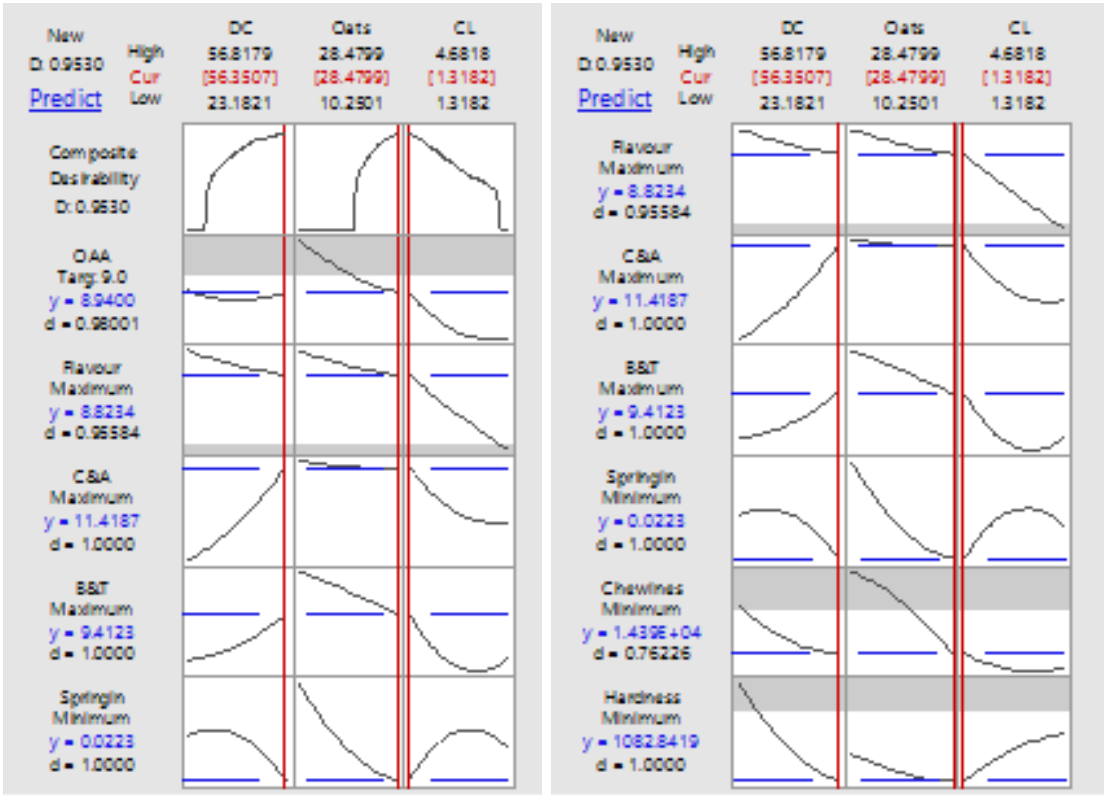


Fig. 4.1 Optimisation graph

Analysis of Textural parameters

Hardness

The hardness of the product was found to be significantly affected by the dark chocolate and oat powder. The minimum and maximum scoring samples are 19 and 10 respectively. In statistical analysis, the hardness can be obtained as below

Hardness = 4916 - 120.0 DC + 18.2 Oats - 391 CL + 1.298 DC*DC + 1.49 Oats*Oats - 70.3 CL*CL - 2.36 DC*Oats + 11.79 DC*CL + 16.7 Oats*CL

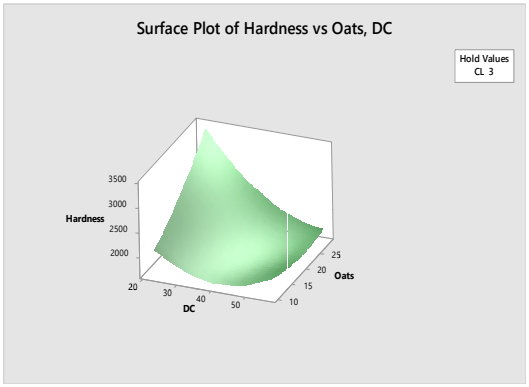


Fig. 4.2(a)

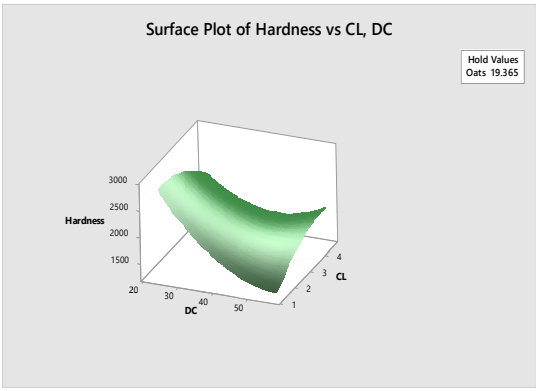


Fig. 4.2(b)

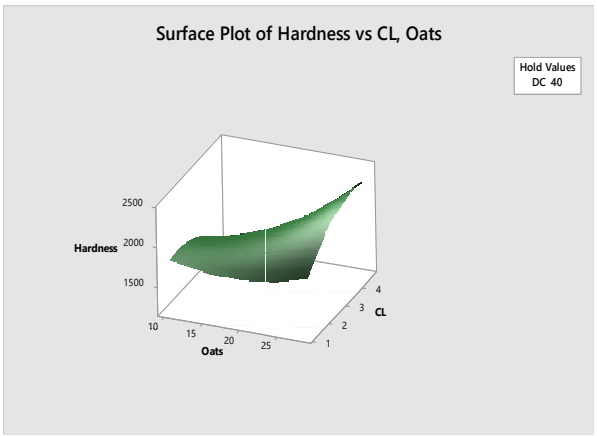


Fig. 4.2(c)

Chewiness

The chewiness of the product was found to be significantly affected by oat powder. The minimum and maximum scoring samples are 16 and 4 respectively. In statistical analysis, the chewiness can be obtained by as given below

Chewiness = -100296 + 2342 DC + 11543 Oats - 20475 CL + 40.2 DC*DC - 118 Oats*Oats + 3304 CL*CL - 219.9 DC*Oats - 518 DC*CL + 917 Oats*CL

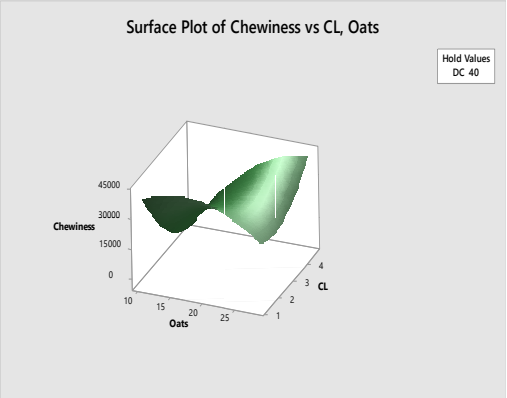


Fig. 4.3(a)

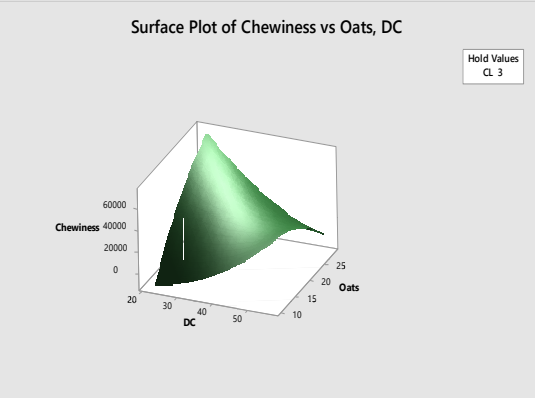


Fig. 4.3(b)

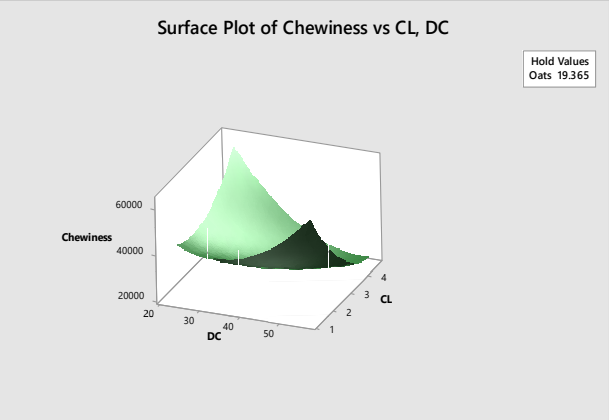


Fig. 4.3(c)

Springiness

The springiness of the product was found to be significantly affected by oat powder. The minimum and maximum scoring samples are 16 and 9 respectively. In statistical analysis, the springiness can be obtained by as given below

Springiness = -0.95 + 0.0628 DC - 0.029 Oats + 0.452 CL - 0.000577 DC*DC + 0.00156 Oats*Oats - 0.0807 CL*CL - 0.00110 DC*Oats + 0.00408 DC*CL - 0.0047 Oats*CL

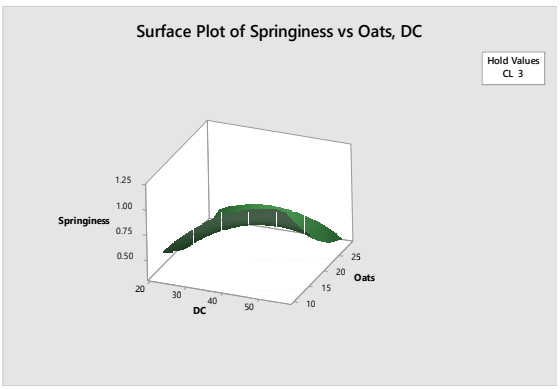


Fig. 4.4(a)

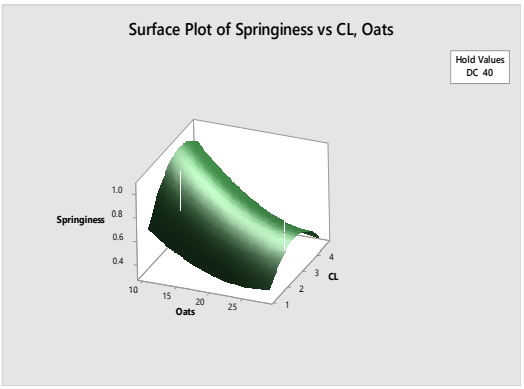


Fig. 4.4(b)

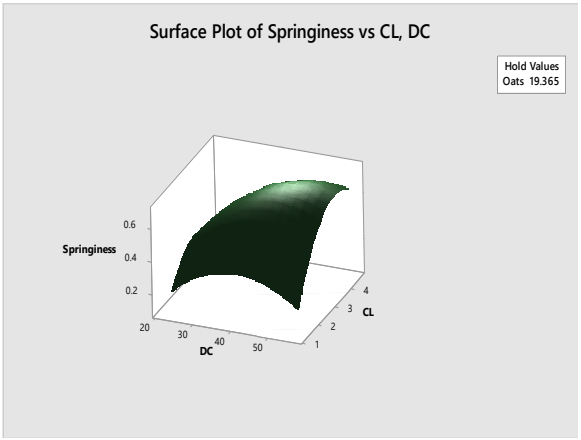


Fig 4.4(c)

Analysis of Sensory parameters

Body and Texture

Body and texture is greatly affected by the amount of dark chocolate and especially by the amount of oats which impart a different body and texture to the dark chocolate compound.

The maximum score was recorded in samples 4,13 and 19 whereas it was minimum for sample no.10. In the statistical analysis, the body and texture can be given as

BandT = 9.16 + 0.023 DC + 0.082 Oats - 2.25 CL + 0.00150 DC*DC - 0.00077 Oats*Oats + 0.503 CL*CL - 0.00227 DC*Oats - 0.0125 DC*CL - 0.0227 Oats*CL

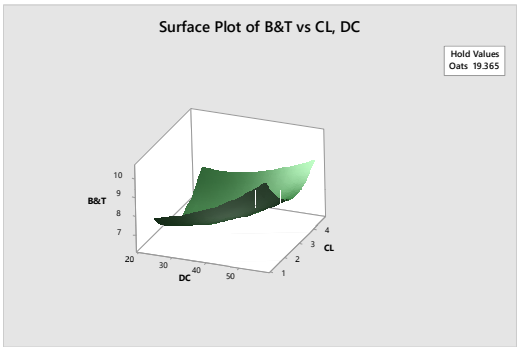


Fig. 4.5(a)

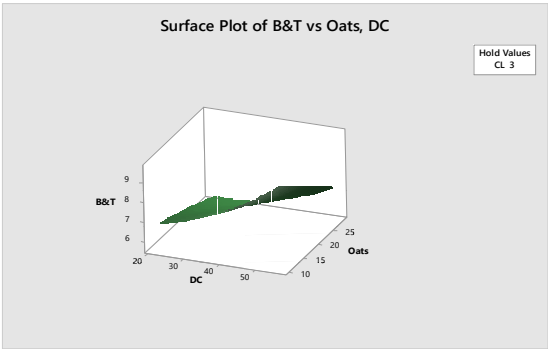


Fig. 4.5(b)

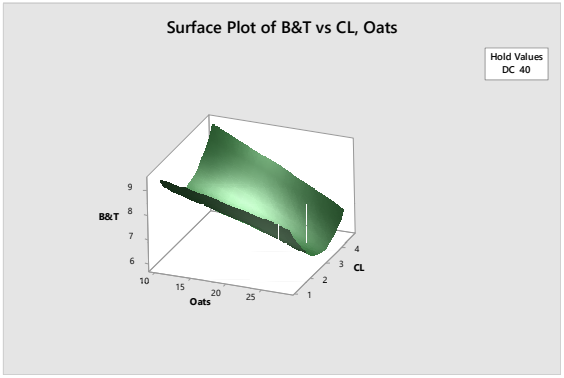


Fig. 4.5(c)

Colour and appearance

Colour and appearance significantly vary with the amount of dark chocolate and oats. Curry leaves do not have a significant effect on this parameter. Colour and appearance was recorded maximum in samples 4, 5 and 9. The minimum values were noted in sample 10. The values of colour and appearance can be calculated using the following equation

CandA = 15.22 - 0.106 DC - 0.334 Oats - 1.86 CL + 0.00213 DC*DC + 0.00151 Oats*Oats + 0.390 CL*CL + 0.00455 DC*Oats - 0.0250 DC*CL + 0.0000 Oats*CL

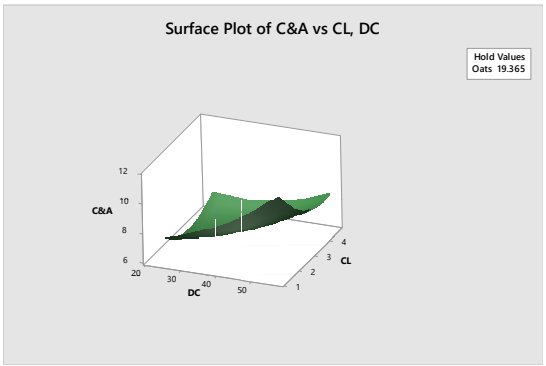
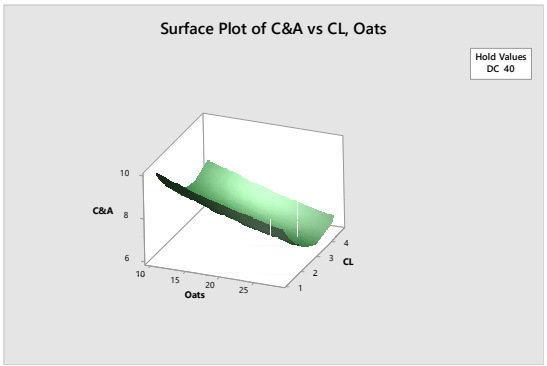


Fig. 4.6(a)



Fig, 4.6(b)

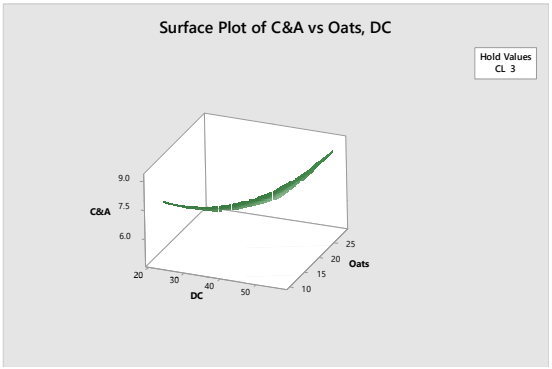


Fig. 4.6(c)

Flavour

The amount of dark chocolate and oat imparts good flavour. However curry leaves powder, even in a small quantity affect this quality significantly. The minimum value for flavour was recorded in sample 8 wand the maximum in sample(s) 4 and 19The values of flavour can be calculated using the following equation

Flavour = 9.13 + 0.0466 DC + 0.110 Oats - 1.540 CL + 0.000526 DC*DC + 0.00184 Oats*Oats + 0.0526 CL*CL - 0.00455 DC*Oats - 0.00000 DC*CL + 0.0000 Oats*CL

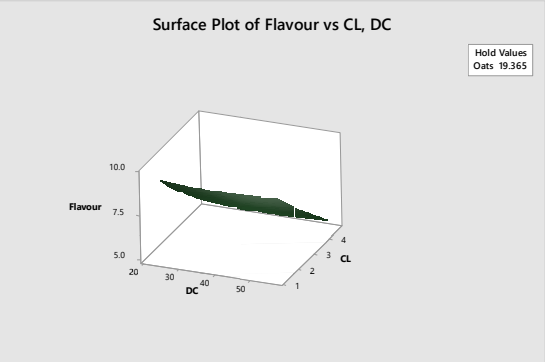


Fig. 4.7(a)

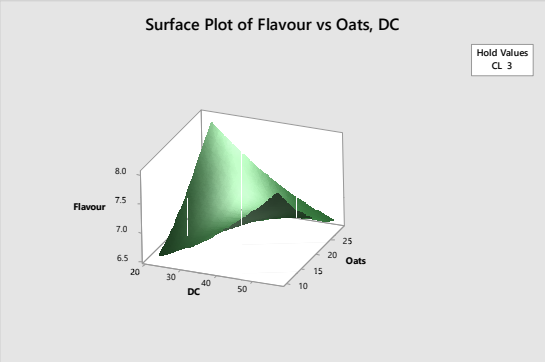


Fig. 4.7(b)

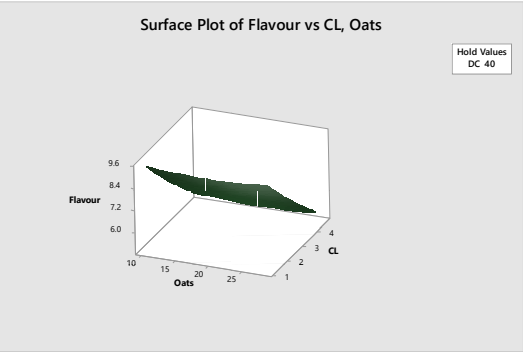


Fig. 4.7(c)

Overall Acceptability

This was measured on overall perception of other factors to conclude the best and optimum sample(s).The minimum levels were recorded as 6 in samples 8, 10 and 12 whereas the maximum overall acceptability was recorded as 9 in sample 4. The values of overall can be calculated using the following equation

OAA = 11.48 + 0.031 DC - 0.055 Oats - 2.45 CL + 0.00122 DC*DC + 0.00426 Oats*Oats + 0.298 CL*CL - 0.00455 DC*Oats - 0.0000 DC*CL + 0.0000 Oats*CL

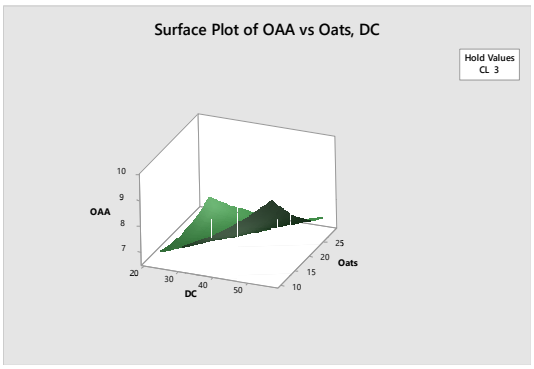


Fig. 4.8(a)

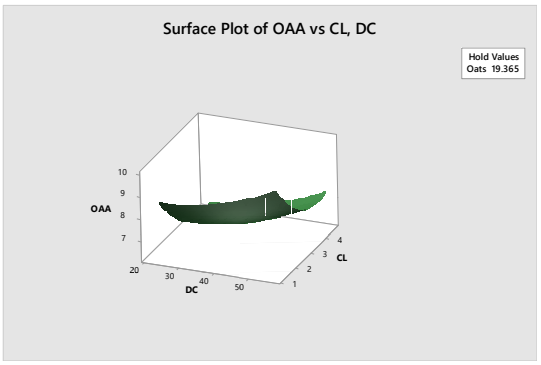


Fig. 4.8(b)

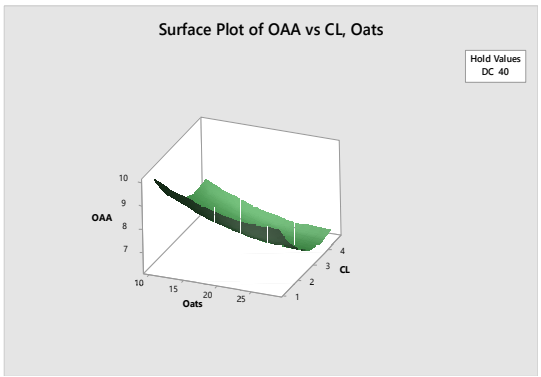


Fig. 4.8(c)

During optimisation of fortified dark chocolate, several response variables were selected that describe the quality characteristics that are in general supposed to be optimised on the basis of selected independent parameters. The optimisation was applied for selected range of dark chocolate (30-50g), oat powder (14-25g) and curry leaves powder (1-4g).

In this study, the level of curry leaves was maintained to be minimised to keep the taste and flavour of dark chocolate considerable and not to deviate from characteristic dark chocolate feel and perception. The purpose is to incorporate average amount of oats and minimum amount of curry leaves without causing severely significant changes in the dark chocolate as present in general.

4.4 Optimised product

The solution was obtained for optimized fortified dark chocolate condition having value as: dark chocolate g, oat powder g and curry leave powder g. The sugar amount was maintained at 8% and g of powdered sugar was used.

Table 4.2 Constituents of functional dark chocolate

Dark chocolate	Oat powder	Curry leaves powder	Sugar
65.4108937gm	32.90899 gm	1.53014177	8 gm

Composition fortified dark chocolate

Dark chocolate - 65.4108937gm /100gm

Roasted ground oats – 32.90899 gm/l00gm

Curry leaves powder - 1.53014177 gm/100gm

Sugar(8%)-8 gm /100gm

4.5 The proximate analysis

Table 4.3 Proximate analysis responses for the product are presented in

TEST	RESULTS
Moisture	94.32
Ash	3.56
Reducing Sugar	3.59
Non-reducing Sugar	31.22
Total Sugar	34.81
Fat	21.43
Protein	8.76
DPPH	3.12
TPC	0.46

4.6 Microbial analysis

Table 4.4 Microbial count in the fortified dark chocolate

MICROBIAL COUNT	CFU/g
Total Plate Count (TPC)	1.12±0.02
Coliform Count	Nil
Yeast and Mould	2.56±0.03

4.7 Studies on cost of production of fortified dark chocolate

The cost of production of fortified dark chocolate was calculated by taking into account the cost of all the ingredients, processing and package material cost. The cost of marketing and distribution together with profit margins are given below.

Table 4.5 Cost of raw material used

INGREDIENT	QUANTITY (g)	PRICE (Rs.)
Amul Dark Chocolate	150	100
Kellog's Oats	500	98
Curry Leaves	500	50
Sugar	500	24

Table 4.6 Cost of production per 100g

ITEM	QUANTITY USED IN MAKING CHOCOLATE (g)	PRICE (Rs,)
Amul Dark Chocolate	65.4108937	43.6072625
Kellog's Oats	32.90899	6.45016204
Curry Leaves	1.53014177	0.153014177
Sugar	8	0.384
Subtotal cost		**50.5944387**
Processing cost @10% of ingredient cost		5.05944387
Packaging cost (wrapping)		10.00
Cost of 100g packaged product		65.6538826
Marketing and distribution expenses expenses @ 25% of packaged product		16.4134706
Cost of 100g packaged product		82.0673532
Profit margin(30% of total cost)		24.620206
Grand Total		**106.687559**

From the data it is calculated that in the preparation of 100g fortified dark chocolate, the total cost of Rs.106.687559 incurred.

4.8 Storage study of optimized fortified dark chocolate

The optimized dark chocolate sample was stored at two different temperatures (4°C and 25°C). Prior to storage, chocolate was wrapped properly in chocolate wrapping paper and kept inside the polyethylene bag. The stability of the optimized

chocolate was studied based on sensory and physical parameter like, colour and appearance, taste, flavor and body and texture.

The change in sensory parameters were measured by the semi trained sensory panel using 9 point hedonic scale

4.8.1 Effect on Color and Appearance

The sensory score of color and appearance decreased linearly in the chocolate samples at temperature 25°C and decrease in color was observed continuously up to one and a half month.

No visible color and appearance was visualized and observed in samples stored at 4°C upto 35 days and thereafter, slight change in color was visible

4.8.2 Effect on flavour and taste

The sensory score of flavour and taste decreased linearly in the chocolate samples at temperature 25°C and decrease in taste was observed continuously up to one and a half month.

Significant change in these samples was noted and observed in samples and the ones stored at 4°C began to show slight change after 35 days and thereafter, slight change in taste was noted.

4.8.3 Effect on Body and Texture

The samples stored at 25°C began to show degradation in body and texture from day 14 and were damaged significantly by the day 28 whereas the samples stored at 4°C showed results even after day 35.

4.8.4 Effect on overall acceptability

The samples stored at 25°C were not appreciated after day 28 whereas the ones at 4°C continued to be fit after 42 days of storage.

4.9 Microbial analysis

Table 4.7 Microbial count in product during storage study

DAY	STORAGE TEMPERATURE	TPC (CFU/g)	Coliform	Yeast and Mold (CFU/g)
0	4°C	1.12±0.02	Nil	2.56±0.03
	25°C	1.12±0.02	Nil	2.56±0.07
7	4°C	1.14±0.03	Nil	2.57±0.02
	25°C	1.14±0.05	Nil	2.59±0.04
14	4°C	1.15±0.02	Nil	2.59±0.03
	25°C	1.17±0.04	Nil	2.61±0.02
21	4°C	1.16±0.05	Nil	2.60±0.05
	25°C	1.21±0.01	Nil	2.63±0.01
28	4°C	1.19±0.02	Nil	2.62±0.04
	25°C	1.26±0.05	Nil	2.67±0.02
35	4°C	1.23±0.06	Nil	2.63±0.03
	25°C	1.31±0.02	Nil	2.75±0.05
42	4°C	1.24±0.03	Nil	2.65±0.02
	25°C	1.33±0.02	Nil	2.83±0.06

Chapter-5

SUMMARY AND CONCLUSION

The present study was undertaken to develop the technology for manufacture of oats and curry leaves fortified dark chocolate bar. The process development involves the drying methods. The proximate compositions of product and drying methods such as sun and shade drying and packaging material (low density polyethylene) on the composition and keeping quality of the product.

The fresh curry leaves were cleaned, sun-dried and powdered. The oats were roasted and grind. Dark chocolate was melted and curry leaves and oats in powdered form were added along with powdered sugar. Different trials were taken for the optimisation process of the product and selection was made on the basis of sensory analysis.

The effects of packaging material on the physio-chemical properties and keeping quality of the chocolate bar were observed at 7 days interval up to 45 days storage period at room temperature (25-30˚C) and lower temperature of 4˚C.

During the storage period of 45 days, the different changes in the product and product content were noted and analysed to conduct shelf-life study of the product. Various physical, chemical and microbiological changes were seen and measured. The product was checked for tempering, oxidation, rancidity, surface microbial growth etc.

The scores for sensory attributes - all decreased with time and with the sample stored at refrigeration temperature showing negligible to minimal changes whereas significant changes regarding degradation in perception of sensory attributes was noted in the samples stored at 25˚C.

In the flavour and taste estimation, it was found that the dark chocolate and oats exhibited a positive effect on the flavour. Flavour imparted by low level of curry leaves was appreciated and that the level of curry leaves had a negative effect on the taste. Only the level of dark chocolate and oats exhibited the positive effect. Due to high level of dark chocolate, taste of increased.

Oats and curry leaves added a diiferent taste. The sensory score of taste decrease linearly in the chocolate sample stored at 25℃. No change in taste was observed in chocolate at 4℃upto one month of storage. The degradation in taste at 25℃ was observed within 28 days as it commenced from 14 days while at 4℃, it commenced after 35 days. Slight change in flavour was observed in chocolate up to one month of storage at temperature 4℃. Flavour decreased linearly in the chocolate sample at temperature 25℃ and the decrease in flavor was observed continuously up to one month.

In the Hardness estimation of' chocolate, it was found that oat exhibited a positive effect on hardness while negative coefficient of dark chocolate and curry leaves had a negative effect on hardness of fortified dark chocolate. Oats and curry leaves were found to be highly hygroscopic in nature and absorbed moisture and became harder.

Also, it was found that dark chocolate exhibited a positive effect on stickiness, while negative coefficient of oats and curry leaves powder showed no significant effect on stickiness of the product.

Optimization of levels of dark chocolate, oat and curry leaves powder. The numerical optimization was carried out by Minitab 17 by applying desirability function method. 1 solution was obtained for the optimum covering criteria with a highest desirability – the sample with lowest concentration of curry leaves . Under these circumstances, the sugar constituent was kept constant at 8 %. The solution was obtained for optimized product condition by incorporation of values as dark chocolate -60.98 gm /100gm, powdered oats –29.72 gm/l00gm, curry leaves powder -1.98 gm/100gm and sugar (8%)-7.32 gm /100gm

Storage study

Optimized chocolate sample was stored at two different temperatures (4°C and 25°C). Stability of the optimized chocolate was studied based on sensory parameter like Color and appearance, taste, flavour, Consistency and texture parameter like Hardness, Stickiness and Stringiness as well as microbial growth.

The changes in pH and titrable acidity depend upon the different types of reactions occurring in the product. This can also be attributed partly to the inherent acid naturally present in the different constituents, mainly *Murraya koenigii.*

Effect on Microbial count

The microbial growth increased with storage period. Microbial analysis was done at 7 day interval. The samples at 4°C were free from contamination or showed negligible and acceptable limits of microbial population while in the samples stored at 25˚C, microbial contamination began to show up early from day 14 and continued to grow further.

Optimized chocolate sample was stored at two different temperatures (4℃ and 25℃). During storage, Total Plate Count (TPC) f'rom 1.12±0.02 to1.24±0.03 and 1.12±0.02 to 1.33±0.02 cfu/g was founded at 4 and 25℃ respectively. The *Coliform* count was found nil during storage period. During storage period total yeast and mold count from 2.56±0.03 to2.65±0.02 and 2.56±0.07 to 2.83±0.06 cfu/g was found at 4 and 25℃ respectively.

The research study conducted concludes that the Oat and Curry leaves - in powdered form were successfully added to make fortified chocolate. The textural characteristics like colour, flavour, taste, consistency and hardness were acceptable. However, the amounts of oats and curry leaves are to be kept comparitively low to maintain the dark chocolate essence. The chocolate was found to be rich in protein, taste, flavour, texture, acceptance and has good shelf life at low temperature 4℃. Due to presence of oats and curry leaves market value of the dark chocolate can also be increased as both the ingredients have various promising health benefits..

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APPENDIX-I

Sensory Evaluation of oats and curry leaves fortified dark chocolate

Name **:** …….. Date……. Time : ……

Dear sir/madam, Please taste these samples and check how much you like and dislike each of on four sensory attributes such as Colour and appearance, Body and texture, Flavour and taste and overall acceptability.

You are next given a number in each sample to define the degree of preference to use the appropriate scale to show your attribute by checking at the point that best describe your feeling about the samples and the quality according to the following scale.

Like Extremly-9, Like Very much-8, Like Moderately-7, Like Slightly-6, Neither Like or Dislike-5, Dislike Slightly-4, Dislike Moderately-3, Dislike Very Much-2, Dislike Extremely-1

Sample code	Colour and appearance	Body and texture	Flavour and taste	Overall acceptability
1				
2				
3				
4				

REMARKS : SIGNATURE

APPENDIX-II

Nutrition Facts - Calories in Amul Dark chocolate Dark

Servings: per 100gm

Cocoa 55%

Calories	561	**Sodium**	0 mg
Total Fat	33 g	**Potassium**	0 mg
Saturated	0 g	**Total Carbs**	59 g
Polyunsaturated	0 g	**Dietary Fiber**	0 g
Monounsaturated	0 g	**Sugars**	43 g
Trans	0 g	**Protein**	7 g
Cholesterol	0 mg		
Vitamin A	0%	**Calcium**	0%
Vitamin C	0%	**Iron**	0%

APPENDIX-III

NUTRITIENT CONTENT OF Oats (per 100g)

Calories : 389

NUTRIENT	VALUE	
Cholesterol	0mg	0%
Protein	17g	34%
Fat	Total fat 7g	10%
	Saturated fat 1.2g	6%
	Polyunsaturated fat 2.5g	
	Monounsaturated fat 2.2g	
Carbohydrate	66g	22%
Calcium	5%	
Iron	26%	
Magnesium	44%	
Vitamin A	0%	
Vitamin B-6	5%	
VitaminB-12	0%	
Vitamin C	0%	
Vitamin D	0%	
Dietary fiber	11g	44%
Sodium	2mg	0%
Potassium	429mg	12%

APPENDIX-IV

Nutritient content of Dehydrated Curry Leaves (Per 100g)

NUTRIENT	VALUE (g)
Protein	12
Fat	5.4
Carbohydrate	64.31
Calcium	2040mg
Iron	12mg
B-carotene	5292µg