ENCAPSULATION OF BLENDED ESSENTIAL OILS AND ITS INCORPORATION FOR STORAGE STABILITY OF CHEVON PATTIES

Thesis

Submitted to the Guru Angad Dev Veterinary and Animal Sciences University in partial fulfilment of the requirements for the degree of

MASTER OF VETERINARY SCIENCE
in
LIVESTOCK PRODUCTS TECHNOLOGY
(Minor Subject: Veterinary Public Health and Epidemiology)

By

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CERTIFICATE – I

This is to certify that the thesis entitled, "**ENCAPSULATION OF BLENDED ESSENTIAL OILS AND ITS INCORPORATION FOR STORAGE STABILITY OF CHEVON PATTIES**" submitted for the degree of M.V.Sc. in the subject of Livestock Products Technology (Minor Subject: Veterinary Public Health and Epidemiology) of the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, is a bonafide research work carried out by **Patil Dheeraj Sunil (L-2019-V-25-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE – II

This is to certify that the thesis entitled, "**ENCAPSULATION OF BLENDED ESSENTIAL OILS AND ITS INCORPORATION FOR STORAGE STABILITY OF CHEVON PATTIES**" submitted by **Patil Dheeraj Sunil (L-2019-V-25-M)** to the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, in partial fulfilment of the requirements for the degree of **M.V.Sc**. in the subject of Livestock Products Technology (Minor Subject: Veterinary Public Health and Epidemiology) has been approved by the Student's Advisory Committee after an oral examination on the same, in collaboration with an external examiner.

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ABSTRACT

The present study was conducted to standardize the process protocol for spray drying encapsulation of blended rosemary and oregano essential oil. Maltodextrin and Gum Arabic were used as wall materials for the experiment in three different combinations viz. 2:1 Maltodextrin: Gum Arabic, 1:2 Maltodextrin: Gum Arabic and 1:1 Maltodextrin: Gum Arabic at three inlet temperatures (150°C, 170°C and 190°C). On the basis of *in-vitro* antimicrobial and anti-oxidant efficacy, concentration of rosemary essential oil (2.5%w/v), oregano essential oil (2.0% w/v) and Tween-80 (1%) was selected for blending followed by encapsulation through spray drying. Nine different combinations, varying wall materials and inlet temperatures were tried and the encapsulated powder was assessed for encapsulation efficiency, residual moisture content, wettability, solubility, bulk and tapped density. On the basis these parameters, combination of MD+GA(2:1) as wall material, at 18% concentration and 170°C inlet temperature was selected as spray drying conditions and parameters for encapsulation. The selected powder was incorporated into goat meat emulsion at three different levels (0.5, 1 and 1.5%) replacing lean chevon in formulation. On the basis of physico-chemical, sensory and instrumental colour analysis, chevon emulsion incorporated with 1% blended essential oil powder was found most suitable for the development of chevon patties. The effect of incorporated powder on different physico-chemical, microbiological and sensory parameters of chevon patties was investigated under aerobic packaging conditions at 4 ± 1°C for 35 days and samples were drawn at 7 days interval. In treated groups, all the physicochemical parameters viz. pH TBARS, FFA, peroxide value and microbial counts were significantly lower ($P < 0.05$) as compared to control and the best results were depicted by encapsulated blended essential oil group. It was concluded that spray drying encapsulation of blended essential oil protected its active components and its incorporation is very useful to control the oxidative and microbial quality of chevon patties during storage for 35 days under refrigeration (4 ± 1°C).

Keywords: Blended essential oil, Encapsulation, spray drying, storage, chevon patties

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Signature of Major Advisor Signature of Student

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ABBREVIATIONS USED

A.B.T.S	:	2-2-azinobis-3 ethylbenthiazoline-6-sulphonic acid radical activity
BHA	:	Butylated Hydroxy Anisole
BHT	:	Butylated Hydroxyl Toluene
CAR	:	Carvacrol
CIN	:	1,8-cineole
CLE	:	Cemba leaf extract
D.P.P.H	:	1,1-diphenyl-2-picrylhydrazyl radical scavenging activity
DMRT	:	Duncan's Multiple Range Test
EE	:	Encapsulation efficiency
EOs	:	Essential oils
FDA	:	Food and Drugs Administration
FFA	:	Free fatty acid
FFA	:	Free fatty acids
FICI	:	Fractional inhibitory concentration index
GA	:	Gum Arabic
GC/MS	:	Gas chromatography/mass spectrometry
GRAS	:	Generally Recognised as Safe"
LDPE	:	Low density polyethylene
LP	:	Lipid peroxidation
MAP	:	Modified Atmospheric Packaging
MBC	:	Minimum bactericidal concentration
MD	:	Maltodextrin
Meq	:	Miliequivalent
Mg	:	Milligram
MIC	:	Minimum inhibitory concentration
ml	:	Millilitre
mm	:	Millimetre
MT	:	Million tonnes

OD	:	Optical Density
OEO	:	Oregano Essential Oil
PET	:	Polyethylene Terephthalate
ppm	:	Parts per million
PSOP	:	Pitaya seed oil powder
PV	:	Peroxide Value
Pvt.	:	Private
REO	:	Rosemary Essential Oil
RSC	:	Radical scavenging capacity
SEM	:	Scanning electron microscope
SPC	:	Standard plate count
SPSS	:	Software Package for Social Sciences
TAA	:	Total antioxidant activity
TBA	:	Thiobarbituric acid
TBARS	:	Thiobarbituric Acid Reactive Substances
TBHQ	:	Tertiary butyl hydroxy quinine
TCC	:	Total carotenoid content
Temp.	:	Temperature
TEO	:	Tarragon essential oil
TEO-NPs	:	TEO-loaded nanoparticles
TVB-N	:	Total volatile basic nitrogen
TVC	:	Total viable count

CHAPTER - I

INTRODUCTION

With an increasing urbanization, the way of life of people in developing countries like India has changed drastically with a consequent increasing demand for safe and hygienic livestock products, particularly meat. Further increasing income has changed the food consumption pattern with expanding focus on processed products (Pandey et al., 2020). Meat is considered as a supreme source of essential nutrients, particularly high-quality protein. Out of all the meats, chevon (goat meat) is extremely well known and broadly preferred by consumers in India and abroad. It has characteristic texture, colour, and taste which makes it more acceptable, and additionally it doesn't confront any strict religious restrictions or taboos like beef and pork for utilization. Its relatively higher muscle to fat ratio, leanness and lower cholesterol content makes it a healthful product for a variety of consumers (Mazhangara, I et al., 2019). In general, the nutrient composition of meat makes it an ideal environment for the growth and multiplication of spoilage microorganisms and food-borne pathogens. It is considered to be a perishable entity right from the processing of meat products to their distributing channels. Prominent issues related to meat quality deterioration are its oxidation reactions leading to decline in its nutritional quality, change in color, texture deterioration, off-odors and off-flavors, and production of other toxic compounds.

Min B & Ahn D U (2005). During lipid oxidation process, the unsaturated fatty acid portion of membrane phospholipids gets oxidized and hydroperoxides are formed which are further susceptible to oxidation or decomposition to secondary oxidation products e.g. short-chain aldehydes, ketones, and other oxidized mixtures that may unfavourably influence the general quality and acceptability of meat.

Application of antimicrobials and antioxidants in fresh and processed meat and meat products to prevent lipid oxidation, for delaying the advancement of off-flavours, and improvement of colour stability, is a common defensive mechanism. In the food industry, these additives can be of both natural and synthetic origins with increase in consumer awareness about their health and well-being, negative perception towards antimicrobials and synthetic preservatives has led to extensive search for natural sources. Synthetic polyphenolic antioxidants such as butylated

hydroxy anisole (BHA), butylated hydroxyl toluene (BHT) and tertiary butyl hydroxy quinine (TBHQ) are added during processing and storage of meat in order to develop shelf stable meat products and also to slow down lipid oxidation in them (Kumar et al., 2015). However, consumer concerns about the risks and safety of synthetic antioxidants in foods has led to the utilization of natural antioxidants in meat and meat products as a substitute to synthetic antioxidants to preserve the meat and meat products, with supplementary health benefits (Fernandes et al., 2018). Further, there have been studies carried out on synthetic antioxidants which confirms their toxicological and carcinogenic effects after long term consumption. Notwithstanding, in view of the concern over the safety of these synthetic mixtures, extensive work is being done to discover novel and naturally available compounds to postpone the oxidative degradation of lipids and keeping up with the nutritional value of foods, including meat (Johnston et al., 2005; de Ciriano et al., 2010). In this context, essential oils and extracts of medicinal herbs and spices have attained value due to the inherent antimicrobial as well as antioxidant properties. Nowadays different meat products are being prepared by using spices, plant extracts, flavours, and essential oils having antioxidant and anti-microbial properties.

Essential oils (EOs) are naturally derived aromatic liquids, with a wide range of biological activities (El Asbahani et al., 2015). They have largely been used as flavouring additives, medicines, or cosmetics (Dima and Dima 2015) and even as insecticidal, antioxidant, anti-inflammatory, anti-allergic, and anticancer agents (Seow et al., 2014). However, many EOs exhibit strong antibacterial, antiviral, and antifungal activities, proving to be a potent source for natural antimicrobials in food and beverage products (Burt, 2004). Oregano (*Origanum vulgare*) essential oil is one of the popular oil that may be used as a preservative in meat. Two major phenols in oregano viz. carvacrol and thymol, constitute about 78-82% and are responsible for most of the antioxidant activity (Yanishlieva et al., 1999). The mixture of aromatic compounds of volatile nature exerts different biological actions, like antimicrobial, anti-inflammatory, and antioxidative, etc. (Bakkali et al., 2008). Rosemary (*Rosmarinus officinalis)* is a small evergreen bush, which belongs to the *Labiatae* family. It is a popular herb having proven strong antioxidant and antimicrobial activities in addition to several other beneficial activities. It is reported to be very active against a wide range of microorganisms Burt (2004).

A major limitation in using essential oils as natural preservatives is that when applied singly and in lower concentrations in the meat matrix, they are not effective enough against a range of organisms and even in exerting antioxidant action. When added in sufficient amounts to produce the desired effect, there is a negative influence on sensory quality. Exploiting synergies between several compounds have been suggested as an answer to the present problem and could be a relatively novel area for research. In addition, the synergistic combinations may even help in reducing the total concentration to be used, which may eventually conceal the adverse effects on sensory attributes. Another major challenge encountered in using essential oils in the meat model system is that they are prone to thermal degradation and also get inactivated by the interaction with compounds present in the meat matrix, which diminishes their potential effect. Moreover, a relatively higher concentration is required to cause an effect in food products compared to those used during in vitro studies which may probably modify the organoleptic characteristics of any food to which they are added (Sanchez et al., 2014 and Tiwari et al., 2009). Further in higher concentrations, they may pose serious health hazards to consumers.

Encapsulation is a promising technology to protect bioactive compounds from inactivation by environmental conditions and reactions with food components as discussed above. It is defined as a process in which minute particles or droplets are coated or incorporated in homogeneous or heterogeneous matrices, which results in the formation of microparticles with many applications (Gouin 2004). In addition to the protection of active components, encapsulation also ensures their controlled release in the food matrix. Further, it increases the stability of active compounds during storage and also reduces the chances of the interaction between active ingredients of antimicrobial compounds with food components, preventing their inactivation. (Maresca et al., 2016).

There are many ways to encapsulate the active ingredients, primarily chemically or mechanically. An important step in the process of encapsulation is the selection of appropriate encapsulating wall materials and the standardization of processing conditions. The polymers should be chemically compatible, non-reactive with the encapsulating component, and provide the desired coating properties such as strength, flexibility, impermeability, and stability (Maresca et al., 2016). Among different modes of encapsulation, spray drying is the best method to adopt at pilot and

industrial scales (Alves et al., 2014). Spray-drying is a process that involves emulsion preparation, homogenization, and atomization (Rodriguez et al., 2016) using a spray drier. The technology is well established, rather in-expensive, and easily adaptable.

For spray drying, typical wall materials include gum arabic, maltodextrins, hydrophobically modified starch, and mixtures thereof (Gouin 2004). Every wall material has unique properties which may be beneficial for the processor in long run. Gum arabic is regarded as to be an excellent wall material, but its high cost and inadequate availability limit its usage as a material for microencapsulation. Maltodextrin is a hydrolysed starch which is produced by partial hydrolysis of starch with acid or enzymes and has many advantages like relatively inexpensive, neutral aroma and taste, low viscosity at high levels, and better protection against oxidation. However, its low emulsifying capacity and less retention of volatiles limit the usage of this wall material (Souza et al., 2018). Thus, the combination of these wall materials may improve the powders' qualities as no single wall material possesses all the qualities required for an ideal encapsulating agent (Chew et al., 2018). Till now very limited research has been conducted on the encapsulation of blended essential oils for their possible use in meat products and it is seen as a novel area to develop for extending the shelf life of meat products.

In the light of the above discussion, to encapsulate blended essential oils and its incorporation in chevon patties for the extension of shelf life, the present study was conducted with the following objectives:

1. To standardize the process protocols for the encapsulation of blended rosemary and oregano essential oils by spray drying and its quality evaluation.

2. To optimize the incorporation levels of encapsulated blended essential oils in chevon patties and evaluation of it's storage stability at refrigeration temperature ($4 \pm 1^{o}C$).

CHAPTER - II

REVIEW OF LITERATURE

Changing lifestyle and urbanization has led to change the preference of consumers towards minimally processed, ready-to-eat meat products with natural bioactive compounds that act as antimicrobial and antioxidants. In meat industry, it is a major challenge to limit the use of synthetic preservatives that have their residual effects on products and their consumption may lead to serious health concerns. A search for natural alternatives finds a promising solution in form of essential oils as a potent antimicrobial and antioxidant agent.

Essential oils

Essential oils (EOs) have been used in ancient medicine for multiple purposes as evident in historical texts. The increased interest of researchers towards essential oils has been increasing due to their inherent antibacterial, antifungal, and anti-carcinogenic properties. They often have a much stronger smell than the plants they come from and contain higher levels of active ingredients. An increasing application of essential oil in food matrices has been reported by many researchers. These naturally occurring antimicrobial and antioxidant agents are highly complex mixtures of often hundreds of individual aromatic volatile oily compounds, which are extracted from different plant materials, such as leaves, barks, stems, roots, flowers, and fruits. Essential oils are classified as "Generally Recognised as Safe" (GRAS) by the Food and Drugs Administration (FDA), thus they are not harmful, due to their natural origin, are more widely accepted by consumers than "synthetic" agents (Goni et al ., 2009). They can be produced in a number of ways, prominently by steam or water distillation and cold pressing. The primary classifications of essential oil compounds are terpenes and terpenoids. Terpenes are a huge class of hydrocarbons, with various chemical features and biological properties e.g. P-cymene, limonene, terpene, sabinene, and α- and β-pinene, etc. In total, more than 3000 types of EOs are known to mankind, of which only 300 are of commercial interest for application in the food or other related industries. Reliance on essential oil is increasing day by day due to its inherent ability to stop or delay the oxidation of lipids. Several essential oils like rosemary, lemon leaf, basil, oregano, ginger, basilica, balm, coriander, rosemary, and clove have been used as potent antimicrobial agents in fresh meat, ground meat,

seafood, and their packaging/edible films for extension of shelf life (Alfonzo et al., 2017).

Ghavam et al. (2020) had reported that essential oils obtained from vascular plants have been demonstrated to be effective in treating bacterial infections. They have active compounds in their leaves, roots, flowers etc. Studies were authenticated by means of gas chromatography/mass spectrometry (GC/MS) and the antimicrobial properties were assayed by measuring inhibition halos, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The oils showed a significant inhibitory and lethal effect on the Gram-negative bacteria displaying strong antibacterial property.

Nazzaro et al. (2017) studied the antifungal properties of essential oils. The versatility of EO's is enormous as far as inhibition of pathogenic fungi is concerned. Various mechanisms of action of essential oils on fungal cells is by cell membrane disruption, alteration, and inhibition of cell wall formation, dysfunction of the fungal mitochondria, and inhibition of efflux pumps etc.

Ribeiro-Santos et al. (2017) have reported that essential oils have antimicrobial activity that can be used to increase shelf life of food. They can be used either in food or food packaging, but low solubility in water, volatilization and susceptibility to oxidation, limits their use. Therefore, any alternative technology like encapsulation is needed that may enhance its stability and efficiency.

Rafiq et al. (2016) evaluated antioxidant and antimicrobial activities of essential oils of white wormwood, rose-scented geranium and bay Laurel against *Salmonella typhimurium* and *Escherichia coli* O157:H7 on fresh produce and investigated its consumer acceptability. The results showed that essential oil derived from rose-scented geranium showed the highest antimicrobial activity.

Djilani and Dicko (2012) highlighted potential usage of EOs in food preservation, apart from their role in agriculture, pharmaceutical and cosmetic industries. Raut et al. (2014) studied medicinal properties of essential oils from some plant families. They reported them to be effective as antibacterial and antifungal agents along with cancer preventive, anti-mutagenic and antiviral activity. They also

found that combination of two different EOs may result in considerable enhancement of the activity compared to the individual oils.

Mechanism of action of essential oils

Generally, the active components of EOs are reported to inhibit microorganisms through disturbance of the cytoplasmic membrane, disrupting the electron flow, proton motive force, active transport and inhibition of protein synthesis (Ribeiro-Santos et al ., 2017). Most of the studies investigated their use primarily as an antimicrobial agent on bacteria, while less is known about their action on yeast and molds (Hyldgaard et al., 2012). It was reported that the gram-positive bacteria are slightly more susceptible to EO gram-negative ones and it could be due to the fact that the cell membrane of gram-positive bacteria has lipoteichoic acids which may facilitate the penetration of hydrophobic compounds of EOs, whereas the presence of an extrinsic membrane, surrounding the cell wall of gram-negative bacteria limits the rate of diffusion of hydrophobic compounds through the lipopolysaccharide layer. (Rodriguez-Garcia et al., 2016).

Antimicrobial and antioxidant activity of Rosemary oil

Amongst all essential oils, rosemary (*Rosmarinus officinalis* L.) essential oil has higher antioxidant, antimicrobial, fungicidal, and anticancer activity, mainly owing to its terpenes and flavonoids contents (de Oliveira Monteschio et al., 2017). Rosemary (*Rosmarinus officinalis L.)* is a well-known aromatic plant since ancient times and is widely used in many preparations. Fresh and dried leaves are regularly utilized in recipes and traditional medicine. As of now, it has been generally researched as a food additive substance that can be added straightforwardly in food and packaging as an antioxidant and an antimicrobial agent (Petrová, et al., 2013).

Kanth et al. (2018) studied *in-vitro* antimicrobial, antibiofilm and antioxidant efficacy of Rosemary essential oil for potential application in meat products. The oil was tested against four Gram-positive (*Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus* and *Enterococcus faecalis*) and six Gram-negative (*Salmonella enterica serovar Typhi, Escherichia coli, Shigella flexneri, Pseudomonas aeruginosa, Proteus mirabilis* and *Klebsiella pneumoniae*) organisms using zone of inhibition and minimum inhibitory concentration (MIC) estimation. They found a

significant activity of tested oil against all the tested organisms along with potent antibiofilm activity and antioxidant action.

Mohsenabadi et al. (2018) studied the physical and antimicrobial properties of REO encapsulated in chitosan nanogel incorporated in starch-carboxy methyl cellulose film. The REO and nanogel alone had inhibitory effects against *Staphylococcus aureus* (*S. aureus*) and by encapsulation; the inhibitory effect of REO was increased.

Honorio et al. (2015) evaluated the antimicrobial activity of the essential oils (EOs) from *Origanum vulgare* L. (OVEO) and *Rosmarinus officinalis* L. (ROEO), and its major major compounds such as carvacrol (CAR) and 1,8-cineole (CIN), respectively and found minimum inhibitory Concentration (MIC) of both OVEO and CAR was 1.25 μL/mL, while for ROEO and CIN the MIC value was 10 μL/mL against tested *S. aureus.*

Pesavento et al. (2015) studied the effect of Rosemary essential oil combinations against *Staphylococcus aureus* and *Listeria monocytogenes*. He used different combinations of oil (0.5% 1%, & 2% v/w) in minced meat samples along with different pathogenic organisms' inoculations at 4 °C temperature. The raw minces meat sample ended with acceptable colour and cooked minced meat samples attained flavour acceptability at threshold level. The (0.5% & 1%) concentrations of REO interpreted excellent bacteriostatic results for all pathogenically inoculated bacterial samples.

Jiang et al. (2011) studied the composition of rosemary essential oil (REO) by gas chromatography–mass spectrometry (GC–MS). They identified a total of 22 components, which constituted 97.41% of the oil. The major constituents were found to be 1,8-cineole (26.54%) and pinene (20.14%).

Napoli et al. (2010) investigated the qualitative and quantitative composition of the essential oils obtained from wild sicilian rosemary plants (*Rosmarinus officinalis* L.). The essential oils were extracted through hydrodistillation and analysed by GC-MS, identifying 100 compounds comprising more than 96% of the oils. Most highly represented components were monoterpenes, both hydrocarbons and oxygenated: former with a range of 21–68% and the latter with a range of 29–79%.

Yang et al. (2010) evaluated the antioxidant activity and major components of six popular and commercially available herb essential oils, including lavender (*Lavendularan gustifolia*), peppermint (*Mentha piperita*), rosemary (*Rosmarius officinalis*), lemon (*Citrus limon*), grapefruit (*Citrus paradise*), and frankincense (*Boswellia carteri*). The essential oils were analyzed by GC–MS and their antioxidant activities were determined by the free radical-scavenging capacity and lipid peroxidation in the linoleic acid system. The major components of the essential oils of lavender, peppermint, rosemary, lemon, grapefruit, and frankincense were linalyl acetate (28.2%), menthol (33.4%), 1,8-cineole (46.1%), limonene (64.5 and 94.2%), and p-menthanol (34.5%), respectively. Lavender essential oil and limonene had shown the highest DPPH radical-scavenging activity with RC_{50} values of 2.1±0.23% and 2.1±0.04%, respectively. Peppermint essential oil presented the highest ABTS radical-scavenging activity (1.6±0.09). For REO both ABTS and DPPH values were found to be effective

Zaouali et al. (2010) studied the essential oil composition *of Rosmarinus officinalis var. typicus* and *var. troglodytorum* endemic to Tunisia and growing wild in different bio-climates by GC and GC–MS. Further their antimicrobial and antioxidant activity were also evaluated. A variation of the chemical composition attributed to varieties rather than to bio-climates was revealed. Based on zone of inhibition and minimum inhibitory concentration (MIC), a low to moderate antimicrobial activity of oils was revealed against eight tested bacteria.

Romano et al. (2009) studied in vitro antioxidant and antimicrobial activity of methanol rosemary extract along with commonly used food additives such as butylated hydroxyanisole and butylated hydroxytoluene. A synergistic oxidation prevention impact amongst rosemary essential oil and BHT was seen for inhibition of *Escherichia coli* and *Staphylococcus aureus*.

Wang et al. (2008) studied the *in vitro* antioxidant activities of *Rosmarinus officinalis* L. essential oil compared to three of its main components (1,8-cineole, α-pinene, β-pinene). They identified 19 compounds, representing 97.97% of the oil, the major constituents of the oil were described as 1,8-cineole(27.23%), α-pinene (19.43%), camphor(14.26%), camphene (11.52%) and β-pinene (6.71%). The oil and the components were tested for their possible antioxidant activity by means of DPPH

assay and β-carotene bleaching test. In the DPPH test system, free radical-scavenging activity of R. *officinalis* L. essential oil, 1,8-cineole,α-pinene and β-pinene were determined to be 62.45% ± 3.42%, 42.7% ± 2.5%, 45.61% ± 4.23% and 46.21% ± 2.24% (v/v), respectively. In the β-carotene bleaching test system, series concentration of samples were tested to show the antioxidant activities of the oil and its main components, whereas the concentrations providing 50% inhibition (IC_{50}) values of R. *officinalis* essential oil, 1,8-cineole, α-pinene and β-pinene were 2.04% ± 0.42%, 4.05% ± 0.65%, 2.28% ± 0.23% and 2.56% ± 0.16% (v/v), respectively. In general, R. *officinalis* essential oil showed greater activity than its components in both systems.

Bozin et al. (2007) analyzed the essential oils of rosemary (*Rosmarinus officinalis* L.) and sage (*Salvia officinalis* L.) by gas chromatography–mass spectrometry and evaluated their antimicrobial and antioxidant activities. Antimicrobial activity was tested against 13 bacterial strains and 6 fungi, including *Candida albicans* and 5 dermatomycetes. Both essential oils had shown significant antibacterial activity on *Escherichia coli, Salmonella typhi, S. enteritidis,* and *Shigella sonei*. REO also exhibited a significant rate of antifungal activity too. Antioxidant activity was evaluated as a free radical scavenging capacity (RSC), together with the effect on lipid peroxidation (LP). Both essential oils reduced the DPPH radical formation (IC_{50}) 3.82 μg/mL for rosemary and 1.78 μg/mL for sage in a dose-dependent manner. Rosemary essential oil had shown strong inhibition of LP.

Celiktas et al. (2007) examined the antimicrobial activity of the essential oils and methanolic extracts of *R. officinalis* collected from three different regions at four different time intervals of the year against, *Klebsiella pneumonia, Proteus Vulgaris, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus epidermidis, Bacillus subtilis, Enterococcus feacalis,* and *Candida albicans*. The antimicrobial efficacy of the methanolic extracts was tested by the disc diffusion method and for essential oil minimum, inhibitory concentration (MIC) was evaluated. The results presented that the tested bacteria were more susceptible to the essential oil and partially to the methanolic extracts. Moreover the antimicrobial activities of essential oils against the tested bacteria differed which could be due to location and seasonal variations.

Fu et al. (2007) have investigated the antimicrobial activity of the essential oils from Santoyo

Clove (*Syzygium aromaticum* L.) and rosemary (*Rosmarinus officinalis* L.) alone and in combination. The composition of oils was studied by GC/MS. Minimum inhibitory concentrations (MIC) of oils against three gram-negative bacteria, three gram-positive bacteria and two fungi were evaluated. The values for MICs of clove oil and rosemary oil varied from 0.062% to 0.5% (v/v) and 0.125% to 1.0% (v/v) respectively.

Santoyo et al. (2005) studied the chemical composition and antimicrobial activity of through Gas chromatography–mass spectroscopy analysis and by the disc diffusion and broth dilution methods respectively. The main components were α-pinene, 1,8-cineole, camphor, verbenone and borneol, constituting 80% of the total oil. It showed antimicrobial activity against all of the microorganisms tested, with inhibition zones and minimal bactericidal and fungicidal concentration values in the range of 17 to 33 mm and 2.25 to 0.25 mg/ml, respectively. *S. aureus* was found to be the most sensitive bacteria to the rosemary extracts, whereas the least susceptible was *A. niger*..

Antimicrobial and antioxidant activity of Oregano essential oil

Oregano *(Origanum vulgare*) is a species of flowering plant belonging to the mint family (*Lamiaceae).* It is a well-known ornamental plant, with the large number of cultivators. Oregano (*Origanum vulgare*) is a significant herb rich in phenolic compounds. Herbs have been extensively used to extend the realistic usability of food sources too. Even though oregano is a native herb of the Mediterranean countries, such as Italy, Greece and Spain, the plant is successfully cultivated in other parts of the world. Two major phenols in oregano viz. carvacrol and thymol, constitute about 78-82% and are responsible for most of the antioxidant activity (Govaris et al., 2010). It is indicated that the oregano essential oil can be in use as a potential resource of natural antioxidants for the food industry. In various studies and investigations carried out on oregano essential oil, it is found to be effective as an antioxidant and flavouring agent (Loizzo et al., 2009) and inhibitor of lipid oxidation (Handl et al., 2008).

Munekata et al. (2020) studied the addition of plant extracts to meat and meat products to extend shelf-life and health-promoting attributes which involved addition of black pepper, oregano, guarana seeds and drumstick flowers. The study concluded that plant extracts can be used as both ingredient or packaging component during short and long storage periods (such as observed for fresh meat, patties, nuggets and sausages).

Oleynikov et al. (2020) studied the antioxidant and antimicrobial properties of oregano extract (*Origani vulgaris herba L)*. Trimmed beef with 20 % and 30% fat was used and treated with oregano extracts and stored for 12 days at 4 °C. Oregano extract proved to be able to inhibit the oxidation of myoglobin in meat and oxidative stability of the treated samples was much higher than control samples.

Agrimonti et al. (2019) studied the antimicrobial activity of cellulosic pads amended with emulsions of essential oils of oregano, thyme and cinnamon against microorganisms in minced beef meat and concluded that three emulsions containing different percentages of carvacrol, thymol, linalool, and α and β-pinene, significantly reduced the growth of *S. enterica* and *P. putida.*

Hernandez et al. (2017) studied sensory and microbial load attributes of dried meat using oregano essential oil. They reported that OEO in meat was effective in hindering *Salmonella enteritidis* and *Escherichia coli* and a value-added dried meat item obtained by utilizing OEO received a satisfactory and acceptable reaction from consumers.

Kirkpinar et al. (2014) studied the effects of dietary oregano and garlic essential oils on carcass characteristics, meat composition, colour, pH and sensory quality of broiler meat and added essential oils to the diet at 300 mg/kg. It affected breast meat's sensory quality at the start of the storage period, at 15 d and at 30 d of storage, except for appearance, pH and b* value and the study concluded that the positive effect of oregano and garlic oils, alone or in combination, may be commercially interesting.

Khanjari et al. (2013) studied the combined effect of N,O-carboxymethyl chitosan (NOCC) and oregano essential oil (OEO) in extension of shelf life and control of *Listeria monocytogenes* in raw chicken meat fillets. Results showed that

total viable count (TVC) exceeded 7 log cfu/g after day 6 and 10 for control samples and samples treated with OEO, respectively. Samples treated with either NOCC or OEO plus NOCC maintained compairitively lower values.

Viuda-Martos et al. (2010) studied the effects of orange dietary fibre, oregano essential oil and packaging conditions on shelf-life of bologna sausages. Samples with fiber and oil stored in vacuum packaging showed the lowest TBA values and they concluded that orange dietary fibre and oregano essential oil could find a use in the food industry to improve the shelf-life of meat products.

Chouliara et al. (2007) evaluated the combined effect of oregano essential oil and modified atmosphere packaging on shelf-life extension of fresh chicken breast meat, stored at 4 °C and concluded that on the basis of sensory evaluation, shelf-life extension of chicken breast meat increased by 3–4 days for samples containing 0.1% oregano oil, 2–3 days for samples under MAP and 5–6 days for samples under MAP containing 0.1% of oregano oil was attained.

Skandamis et al. (2001) investigated inhibition of *Escherichia coli* O157: H7 by oregano essential oil and EDTA and found that the essential oil, EDTA, and also the incubation temperature of the medium influenced the utmost rate of growth and also the lag phase of Escherichia coli O157: H7. The presence of oregano essential oil at the time of inoculation caused a rise in the lag phase and reduced final population size.

Lambert et al. (2001) studied the minimum inhibitory concentration and mode of action of oregano essential oil, thymol, and carvacrol and stated that mixtures of carvacrol and thymol give an additive impact and is responsible for the overall inhibition by oregano essential oil.

Blending of Essential oils for synergistic effect

As a result of the increasing resistance to antibiotics, the discovery of novel antimicrobial agents is very interesting. Essential oils are widely used as constituents in pharmaceutical products, flavouring agents and fragrances in food and cosmetic industries, and aromatherapy. They have different natural properties, like antibacterial, antifungal, antiviral, insecticidal etc. However, mostly only pure/single essential oils have been tested by many researchers for their antimicrobial activity,

while lesser number of studies on antimicrobial activity of blended essential oil preparations have been carried out.

Oh et al. (2017) studied effect of single essential oil and blend of essential oils (Oregano essential oil and Thyme essential oil) on anti-microbial biofilm against *E. coli* and *Salmonella* strains in *in vitro* experiment. Essential oils vigorously influenced the counter biofilm advancement of *E. coli* and *Salmonella*. In addition, the single Essential oil was having equitable antibiofilm activity over mixed essential oil.

Blending of oils results in hiking the potency and offers a balance and most of the times, these blends would give a synergistic effect on antimicrobial and antioxidant action. The synergistic interactions lead to raising their efficacy at a concentration low enough to avoid adverse effects and thus facilitate their use as food preservation system (Bag & Chattopadhyay 2015).

Rimini et al. (2014) used blends of thyme and orange oil on marinated chicken meat to improve its quality traits. Two replications were done to assess the impact of a combination (1:1) of thyme and orange oils (EO) on the quality attributes and the oxidative stability of chicken meat (breast and wing). It was observed that as compared to single oil combination, blending provided a better solution as far as antimicrobial and antioxidant efficacy is concerned.

Olmedo et al. (2013) utilized blends of rosemary and oregano essential oil on oxidative and fermentative quality of flavoured seasoned cheddar cheese arranged with cream cheddar base. A protective effect was observed in flavoured cheese prepared with cream cheese base against lipid oxidation and fermentation with the use of blends of oregano and rosemary essential oil.

De Oliveira et al. (2012) observed a synergistic effect by combining winter savory essential oil and sodium nitrite. The results offered the possibility of using the combination of essential oils and food additives for reducing the levels of nitrites in meat products.

Mathlouthi et al .(2011) studied *in vitro* antimicrobial activity of a blend of 3 different essential oils (oregno, rosemary, and a commercial blend of essential oils) against pathogenic and non-pathogenic microscopic organisms and to studied their

impacts on Broiler chickenperformances. They found that blends were more effective in different actions rather than a single oil.

Bassolé et al. (2010) showed that if linalool or menthol was combined with eugenol it showed the highest synergy, suggesting that a monoterpenoid phenol combined with a monoterpenoid alcohol is an effective combination.

Goni et al. (2009) studied the antimicrobial activity of the combination of cinnamon and clove essential oils against the growth of four Gram-negative and four Gram-positive bacteria. The assessment was carried out by means of the fractional inhibitory concentration index (*FICI*) of the mixture. If the minimal inhibitory concentrations were applied, the vapours of the combination of essential oils exerted an antagonistic effect on the growth of *E. coli*, while they wielded a synergistic effect for the inhibition of *L. monocytogenes*, *B. cereus* and *Y. enterocolitica* when the concentrations of maximal inhibition were used.

Need for Encapsulation of essential oils

The essential oil and its active principles or its blends have potential use in livestock products industry aiming to maintain/improve meat quality during storage. However, these oils contain bio active compounds which exhibit significant susceptibility to light and temperature. Besides, they are insoluble in water, and for specific applications, a controlled delivery system is required. In this manner, an adequate formulation of the essential oil which considers these perspectives is required for industrial applications. Common goals in the development of essential oil formulations is to protect the essential oil from degradation or from losses by temperature, to achieve a controlled release, and to facilitate handling (Martin et al., 2010). The application of EOs as preservatives in food has also been limited by many other factors. One of the challenging factors is change in sensory characteristics of foods which could be due to relatively high EOs concentrations needed to achieve microbial control. Moreover EOs have strong aroma and taste and even at low concentrations they can bring negative effects on the organoleptic properties of the food. In order to overcome undesirable sensory characteristics lowering the EOs concentration may in turn compromise the effectiveness of the antimicrobial activity of EOs. However, the use of combination of low concentrations of EOs with other

hurdle may result in a synergistic effect without negative organoleptic effects (Sanchez-ortega et al., 2014).

For utilization of EOs in food matrices, its stability is considered as one of the principle factor. Due to complex nature and composition of food products, the effectiveness of EOs and their compounds is largely affected by interactions with other components of food. This poses a major challenge for the use of these EOs. Antimicrobial compounds in essential oil could be subjected to rapid inactivation by binding to food components such as proteins and lipids, or degradation by proteolytic enzymes, resulting in a reduced availability to act against microorganisms in food matrices (Cava-Roda et al., 2012). High fat content in foods is reported to decrease the antimicrobial activity of phenolic compounds. Therefore Encapsulation is seem to be a promising tool to protect bioactive compounds from inactivation by environmental conditions and interactions with food components as discussed above. It is an effective means to enhance biological activity as well as stability of active ingredients in foods and achieve controlled release over time. It has been developed extensively in the last few years to design and formulate procedures for the stabilization, solubilisation, and delivery of the active components in food, pharmaceutical, and cosmetic industries.

Encapsulation by Spray-drying

Spray drying involves atomization of an emulsion or solution in which the atomized droplets get dried in drying chamber, leading to formation of micro particles through thermodynamic phenomena. They are generally collected in a cyclone, and the air exits the system with a lower temperature and a higher humidity. It is a relatively inexpensive, rapid and efficient system for the microencapsulation of active components such as essential oils, natural colorants, vitamins, and probiotics. Proteins, such as dairy proteins and plant protein isolates, and polysaccharides / gums, such as gum arabic, maltodextrin, modified starch, inulin, and cashew gum are the most commonly used wall materials.

Radunz (2020) used casein – maltodextrin as wall materials for preparing spray dried encapsulated thyme essential oil powder. The encapsulated powder quality was assessed by encapsulation efficiency, thermal stability, chemical compounds and morphology. Antioxidant and antimicrobial potential *in vitro* and *in*

situ was assessed by DPPH, hydroxyl and nitric oxide methods in hamburger-like meat product. The casein-maltodextrin encapsulated essential oil showed higher antioxidant and antimicrobial activity against *Staphylococcus aureus, Escherichia coli*, *Listeria monocytogenes* and *Salmonella* Typhimurium tested in vitro and against thermotolerants coliforms and *Escherichia coli* in situ, showing potential for application as a natural preservative in food.

Premi, M., & Sharma, H. K (2017) evaluated the effect of different combinations of wall materials viz. maltodextrin, gum arabic and whey protein concentrate on the encapsulation behaviour and oxidative stability of spray dried drumstick (*Moringa oleifera*) oil. The spray dried oil was characterized for its physical and flow properties, microstructure and oxidative stability. The microcapsules obtained by MD:GA wall material had higher encapsulation efficiency along with medium flow properties and better oxidative stability.

Tolun et al. (2016). investigated the microencapsulation of grape polyphenols using wall materials viz. maltodextrin and gum arabic for enhancing their stability and protecting them from oxidation, light, moisture and temperature. The parameters like yields, hygroscopicity, total and surface phenolic contents, antioxidant activity, individual phenolic compounds and particle morphology were evaluated. The ratio of maltodextrin: gum arabic (8:2) at 140°C drying temperature was considered as the best conditions to get most efficient microcapsules.

Boonchu T. & Utama-ang N. (2015) studied the optimization of extraction and microencapsulation of bioactive compounds from red grape (*Vitis vinifera* L.) pomace by using response surface methodology. The results showed that the optimized microencapsulation used 10.21 % w/v maltodextrin and 0.21 % w/v CMC to maximize all polyphenolic compounds, and also to minimize bitterness and astringency.

Teodoro et al. (2014) microencapsulated rosemary essential oil using spray-drying with modified starch and maltodextrin as wall materials and evaluated its antimicrobial effect on fresh dough. The obtained microcapsules retained 50% of oil after drying with the major oil components being 1, 8-cineole (29.0%), camphor (26.6%), and α-pinene (10.6%). They concluded that through microencapsulation process the antimicrobial property of rosemary essential oil (against *Penicillium sp*.

and *Aspergillus sp*.) was retained and provided this activity for long time when applied to fresh dough.

Botrel et al. (2012) investigated the spray drying conditions on properties of microencapsulated oregano essential oil at different feed rates (L /min) and inlet temperatures (°C). Powder yield was significantly affected ($P < 0.10$) by inlet air temperature and feed rate and with increase in inlet temperature and feed rate higher powder yield was obtained. Regarding retention of oil, the results showed a significant ($P < 0.05$) interaction between the two studied factors.

Lim et al. (2012) studied the effects of different wall materials on the physicochemical properties and oxidative stability of spray-dried microencapsulated red-fleshed pitaya (*Hylocereus polyrhizus*) seed oil. By scanning electron microscope (SEM) the microstructure and morphology of pitaya seed oil powder (PSOP) were observed. PSOP encapsulated with gum Arabic exhibited a lower degree of microencapsulation efficiency compared to PSOP encapsulated with proteinaceous bases.

Tuyen et al. (2010) investigated the effects of spray drying conditions on the physicochemical and antioxidant properties of the (*Momordica cochinchinensis)* fruit aril powder. They studied the effects of different inlet drying air temperature (120°C, 140°C, 160°C, 180°C and 200°C) and maltodextrin addition (10%, 20% and 30%) on the physicochemical and antioxidant properties of the gac aril powder. The moisture content, bulk density, colour characteristics, total carotenoid content (TCC), encapsulation efficiency and total antioxidant activity (TAA) were significantly affected maltodextrin concentration and the inlet air temperatures.

Storage studies of products

Bharti et al. (2020) assessed the shelf of life chicken nuggets wrapped with Carrageenan bio-based composite active functionalized film with anise, caraway, and nutmeg essential oils (EOs). Chicken nuggets overwrapped with aforementioned films were stored aerobically at refrigeration temperature (4 ± 1 °C) for storage period of 15 days. The result reflected that pH, peroxide value , free fatty acid (FFA), and thiobarbituric acid (TBA) value of treatments were significantly ($P < 0.05$) lower than controls. The application of composite, active edible bio-based film was found

proficient in confining product quality attributes throughout storage.

Zhang et al. (2020) studied effects of chitosan-gelatin coatings containing TEO tarragon essential oil or TEO-loaded nanoparticles (TEO-NPs) on the preservation of pork slices during refrigerated storage for 16 days. The outcomes proposed that the coating treatments could significantly inhibit the quality deterioration of pork slices. The Nano-encapsulation method contributed to the slow and steady release of TEO caused further developed antioxidant, antibacterial, and sensory qualities.

Hajrawati H et al. (2019) studied effect of Cemba (*Albizia lebbeckoides* Benth.) leaf extract (CLE) on some of the physical properties, antioxidant and antimicrobial activities, when incorporated into beef patties during cold storage. They concluded that the CLE 1% at was effective to retard lipid oxidation and inhibit bacterial growth of cooked beef patties.

Dong et al. (2018) investigated the characterization and preservation performance of active polyethylene films containing rosemary and cinnamon essential oils for packaging of Pacific white shrimp. Shrimp packaged in active films containing EOs presented lower microbial counts, total volatile basic nitrogen (TVB-N) contents and thiobarbituric acid reactive substances (TBARS) values compared to samples packed in control films during storage at 4 °C for 10 days.

Mohsenabadi et al.(2018) studied the physical and antimicrobial properties of encapsulated rosemary essential oil in chitosan nanogel incorporated in starch-carboxy methyl cellulose film. The CS-BA nanogel incorporated films had a higher water vapor permeability compared with the films containing REO. REO and nanogel alone had shown inhibitory effects against *Staphylococcus aureus* (*S. aureus*) and by encapsulation; enhancement in the inhibitory effect of REO was noticed.

Al-Hijazeen et al. (2016) investigated the effect of oregano essential oil on the oxidative stability and color of raw and cooked chicken breast meats. It was found that, oregano oil at 400 ppm showed the strongest effect for all studied parameters. Hexanal was the major aldehyde, which was decreased significantly ($p < 0.05$) by oregano oil treatment, in cooked meat.

Van Haute et al. (2016) studied the effect of cinnamon, oregano, and thyme

essential oils in the marinade on the microbial shelf life of fish and meat products and concluded that sensorial properties of the meat/fish were inevitably affected when the necessary EO concentrations were added to extend the microbial shelf life are applied.

Kahraman et al. (2015) investigated the effect of rosemary (*Rosmarinus officinalis* L.) essential oil (REO) and modified-atmosphere packaging (MAP) on the survival of certain pathogens in poultry fillets and on their meat quality during 7 days of refrigerated storage and found that in a suitable combination, REO can be applied to improve the quality of meat.

CHAPTER - III

MATERIALS AND METHOD

3.1 SOURCE OF RAW MATERIALS

3.1.1 Source of meat

Goats of age 8-10 months, weighing 30-40 Kg were procured from the Goat Farm, Department of Livestock Production Management, GADVASU, Ludhiana. The goats were slaughtered as per standard procedure in the experimental slaughter house of Department of Livestock Products Technology, College of Veterinary Science, GADVASU, Ludhiana, Punjab with due consideration of the animal welfare aspects. The dressed carcasses were brought to the laboratory immediately and chilled at 4±1ºC for 12-18 hrs and then deboned manually. The skin, external fascia, fat and all separable connective tissues were removed and boneless meat was recovered. The boneless meat and fat were packed separately in low density polyethylene (LDPE) bags in the unit pack of 1 kg and subsequently stored in a deep freezer at -18±1ºC till further use. The required quantity of frozen meat packs were taken out and thawed overnight in a refrigerator (4±2ºC) and portioned into smaller chunks of size, approximately 1 square inch for further study.

3.1.2 Refined vegetable oil

The refined soybean oil (Fortune, Adani Wilmar Ltd) was procured from local market of Ludhiana; Punjab, India was used as added fat in the formulation.

3.1.3 Refined wheat flour (Maida)

Refined wheat flour (Maida) was procured from local market of Ludhiana, Punjab, India was used in formulation as binder

3.1.4 Spice mix

Different spice ingredients were procured from local market, Ludhiana, Punjab. After removal of extraneous matter, the spices were oven dried at 45±2ºC for 2 h. The ingredients were ground mechanically in a grinder (Inalsa, Wondermaxie plus, Delhi, India) and sieved through a fine mesh. The different spice powders were mixed in the standardized proportion (Table 1) to prepare a spice mix. The spice mix was stored in a moisture free Polyethylene Terephthalate (PET) jars till further use.

Table 1: Formulation of Spice Mix

Ingredients	Percentage
Coriander (*Dhania*)	20.60
Cumin seeds (*Zeera*)	15.50
Caraway seeds (*Ajwain*)	10.40
Aniseed (*Soanf*)	12.40
Black pepper (*Kali Mirch*)	12.40
Capsicum (*Mirch Powder*)	10.40
Cinnamon (*Dalchini*)	5.15
Cloves (*Laung*)	2.00
Cardamom large (*Badi Elaichi*)	5.15
Mace(*Javitri*)	2.00
Nutmeg (*Jaifal*)	2.00
Cardamom small (*Chhoti Elaichi*)	2.00
Total	**100**

3.1.5 Eggs

The eggs were procured from University Poultry Farm, Department of Livestock Production and Management, GADVASU, Ludhiana.

3.1.6 Condiment Mix

Condiment paste was prepared from onion, ginger and garlic. The outer layer of onion, ginger and garlic was peeled off and were cut into small pieces and fine condiment paste was made by blending onion, ginger and garlic in a ratio of 3:1:1 in a grinder (Inalsa, Wonder maxie plus, Delhi, India) with a suitable blade.

3.1.7 Salt, Tetra sodium pyrophosphate and Sodium nitrite

The salt used in the study was table salt (Tata Chemicals Ltd., Mumbai India), Tetra sodium pyrophosphate (Hi-media Laboratories Pvt. Ltd., Mumbai India) and Sodium nitrite (Central Drug House Pvt. Ltd., New Delhi, India) were used in product

development.

3.1.8 Packaging Materials

Low density polyethylene (LDPE 100-120 gauge) bags of capacity 1 Kg were used for aerobic packaging.

3.1.9 Chemicals, media and standards

Different analytical grade chemicals, media, and high purity standards required for analyzing the quality of raw and cooked products were procured from standard firms like Sisco Research Laboratories, Fisher Scientific, Central Drug House, Hi-Media and Sigma-Aldrich etc.

3.2 Formulation of chevon patties

Formulation and processing protocols of the chevon patties was standardized on the basis of available literature and various preliminary trails conducted in laboratory. The standardized formulation is given in Table 2.

Table 2: Formulation of the chevon patties

Ingredients	Percentage (w/w)
Lean chevon	64.50
Ice/Chilled water	8.00
Vegetable oil	7.00
Condiments	3.00
Salt	1.50
Refined wheat flour	3.00
Dry spices	2.50
STPP	0.20
Sugar	0.30
Nitrite	100 ppm
Egg	5.00
Soya	5.00

3.2.1 Processing of Chevon patties

The tenderized frozen meat samples were cut into small chunks after partial thawing in a refrigerator and double minced through a meat mincer (Mado Eskimo Mew-714, Mado, Germany) using 4 mm plate. Meat emulsion was prepared in a bowl chopper (Seydelmann K20, Ras, Germany). Pre-weighed quantity of minced chevon, salt, sodium tripolyphosphate and sodium nitrite was added and chopping was done for about 2-3 minutes. It was chopped again for 2 minutes after the addition of ice flakes. Refined vegetable oil was slowly incorporated while chopping till it was completely dispersed in the batter eREO (1%), Condiment paste, dry spice mix, refined wheat flour and other ingredients were added. Chopping was continued till uniform dispersion of all the ingredients and desired consistency of the emulsion was achieved. Meat emulsion of about 25 gm was filled in petri plates. These were kept in oven at 180ºC for 20 min.

3.2.2 Flow diagram for preparation of chevon patties

Frozen chevon at -18±1ºC

↓

Thawing of chevon at 4±1ºC for 24 hrs

↓

Mincing through 4 mm sieve plate

↓

Chopping in bowl chopper with salt, STPP and sodium nitrite with ice flakes

↓

Addition of refined vegetable oil

↓

Chopping again (2-3 minutes)

↓

Addition of spices, encapsulated powder (1%), condiments and other ingredients

↓

Chopping till uniform dough formation

↓

Filling of emulsion in petri plates

↓

Keep in oven at 180ºC for 20 minutes

↓

Cooling, packaging and storage of chevon patties at 4±1ºC

↓

Sensory evaluation and sample analysis.

3.3 Experimental details

3.3.1 Experiment No. 1: Standardization of process protocols for the development of encapsulated blended Rosemary and oregano essential oil by spray drying and evaluation of its quality

Encapsulation through spray drying was done at different inlet temperatures using maltodextrin and Gum Arabic at three concentrations viz. 2:1, 1:2 and 1:1. The parameters that were studied are as under:

3.3.1.1. Encapsulation Efficiency

It was determined according to method suggested by Ton et al. (2016) with slight modifications; the surface oil content of the powder particles (g/g) was determined by a method. One gram of the powder was accurately weighed into the extraction flask. Subsequently, 25mL of petroleum ether (b.p. 40–60°C) was added and the mixture was shook vigorously for 10 min. The mixture was then filtered through a cloth. The filtrate was transferred into the Petri dish, dried at 102±2°C for 1 h and weighed. The surface oil content was calculated as the difference between weight of Petri dish with oil and weight of initial Petri dish. The total oil content of the spray-dried powder (g/g) was determined by using a method described by Ton et al. (2016). One gram of the powder was accurately weighed into the oil extraction flask. Water was added to complete the volume to 10 mL and mixed to form emulsion. Ten milliliters of emulsion was taken into the oil extraction flask for analysis. Firstly, 1.5 mL of ammonium hydroxide was added and mixed followed by 10 mL of alcohol (9%) and the contents were again well mixed. Secondly, 25 mL diethyl ether was added to the flask; it was then shaken vigorously for 1 min. Finally, 25 mL of light petroleum ether (b.p. 40°C –60°C) was added and the flask was shook vigorously for 1 min. After separation was complete, the oil solution was transferred into a Petri dish and the Petri dish was dried at 102±2°C for1 h and weighed. The total oil content was calculated as the difference between weight of Petri dish with oil and weight of initial Petri dish. The encapsulated oil content (g/g) was calculated as a difference of the total oil content and the surface oil content of the powder obtained.

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Total oil content} - \text{Surface oil content}}{\text{Total oil content}} \times 100$$

3.3.1.2. Residual Moisture Content

It was calculated using the method given by Lakshanasomya et al. (2011) by drying at 110±2^{o}C in hot air oven until constant weight.

3.3.1.3. Solubility

It was evaluated according to the method proposed by Cano-Chauca et al. (2005). The powder was weighed (1 g) and stirred in 25 mL of distilled water for 5 min using a blender. The solution was then centrifuged at 3000 × g for 10 min. A 20-mL aliquot of the supernatant was transferred to pre-weighed Petri dishes and oven-dried at 105°C overnight. The solubility (%) was calculated as the percentage of dried supernatant in relation to amount of powder originally added (1.0 gm).

3.3.1.4 Wettability

It was evaluated using the method described by Fuchs et al. (2006). One gram of powder was sprinkled over the surface of 100 mL of distilled water at 20°C without agitation. The time took for the powder particles to become sediment, to sink, or to become submersed and disappear from the water's surface was measured and used in a comparison of the extent of wettability between samples.

3.3.1.5 Bulk Density

It was calculated by using the method of Jinapong et al. (2008). The powders were gently loaded into a 100 mL tared graduated cylinder filled to the 100-mL mark and weighed. The volume read directly from the loaded cylinder was then used to calculate the bulk density (d) according to the mass/volume relationship.

3.3.1.6 Tapped Density

It was evaluated using the method described by de Barros Fernandes et al. (2014). Approximately 5 g of powder was poured into a 25-mL graduated cylinder and then the cylinder was repeatedly tapped by lifting and dropping it under its own weight until a negligible difference in volume between successive measurements was observed. Given the mass (m) and the apparent (tapped) volume (V) of the powder, the powder bulk density was calculated as m/V (g/cm^3)

3.3.2 Experiment 2: To optimize the incorporation levels of encapsulated blended essential oils in chevon patties and evaluation of its storage stability at refrigeration temperature (4 ±1°C).

3.3.2.1 Sub Experiment 2 (a) Optimization of the incorporation levels of encapsulated powder in goat meat emulsion

Three different levels viz. 0.5%, 1% and 1.5% of encapsulated blended essential oil powders (Experiment No. 1) were incorporated separately in goat meat emulsion, replacing the lean meat in the pre-standardized formulation. The formulation of goat meat emulsion is mentioned in Table 3.

Table 3: Formulation of Goat Meat emulsion

Ingredients %	Control	T-1	T-2	T-3
Lean meat	65.50	64.50	63.50	62.50
Ice/Chilled water	8	8	8	8
Encapsulated REO	-	1	2	3
Vegetable oil	7	7	7	7
Condiments	3	3	3	3
Salt	1.50	1.50	1.50	1.50
Refined wheat flour	3.00	3.00	3.00	3.00
Dry spices	1.50	1.50	1.50	1.50
STPP	0.20	0.20	0.20	0.20
Sugar	0.30	0.30	0.30	0.30
Nitrite	100 ppm	100 ppm	100 ppm	100 ppm
Egg	5	5	5	5
Soya	5	5	5	5

The goat meat emulsion was evaluated for emulsion stability, Colour, pH and sensory parameters (8- point descriptive scale). On the basis of these parameters, the final level of incorporation of encapsulated blended essential oil powder in goat meat emulsion was optimized and was used for formulation of chevon patties. There were in total four treatments for the storage study.

3.3.2.2 Experiment No. 2 (b): Evaluation of the storage stability of developed chevon patties incorporated with 1% blended essential oil encapsulated powder at refrigeration temperature (4±1°C).

After processing, the chevon patties were packed under aerobic (LDPE bags) and stored in dark at the refrigeration temperature of 4±1°C. The samples were regularly withdrawn at 7 days interval till 35 days of storage. Storage quality was evaluated on the basis of various quality parameters like pH, Thiobarbituric Acid Reactive Substances (TBARS) number, Free Fatty acids % (FFA), Peroxide Value (PV) and sensory quality attributes. Microbiological quality was evaluated on the basis of enumeration of Standard Plate count, coliforms count, and psychrophilic count. Duplicate samples were taken for each parameter and the experiment was repeated thrice for the consistency of the results**.** There were in total four different treatments for storage studies:

C	Control chevon patties
T_1	Product with spray dried encapsulated rosemary essential oil (4.5%) powder
T_2	Product with spray dried encapsulated oregano essential oil (4.5%) powder
T_3	Product with encapsulated blended rosemary (2.5%) and oregano (2%) essential oil powder

3.3.2.3 Analytical Techniques

3.3.2.3.1 pH

The pH of emulsion and chevon patties was determined as per the method described by Trout et al. (1992) with a digital pH meter (FE-20-1-KIT, Mettler-Toledo India Pvt. Ltd., Mumbai) equipped with a combined glass electrode. Ten grams of sample was homogenized with 50 ml of distilled water for 1 min using pestle and mortar. The electrode was dipped into the suspension and the pH value of the sample was recorded.

3.3.2.3.2 Emulsion Stability

Twenty gram of meat emulsion was taken in low density polyethylene (LDPE) bags of 150 gauge (size 11 × 10 cm) and were placed in a thermostatically controlled

water bath (Equitron, Model: 8414, Medica Instrument Mfg. Co., Mumbai, India) at 80±1°C for 20 min. After that the bags were removed from water bath, drained off the fluid (fat, water soluble solids) and weight of the cooked mass was recorded. The cooked emulsion was weighed and expressed as percentage Baliga and Madaiach (1970).

3.3.2.3.3 Colour profile analysis

Colour profile was measured using Lovibond Tintometer (Lovibond house United Kingdom) set at 2^o of cool white light (d_{65}) and known as '*l*', *a*, and *b* values. '*l*' value denotes (brightness 100) or lightness (0), *a* (+ redness/- greenness), *b* (+ yellowness/- blueness) values were recorded on/in a hundreds of chevon patties kept in a plate. The instrument was calibrated using light trap (black hole) and white tile provided with the instrument. Then the above colour parameters were selected. The instrument was directly put on the surface of chevon emulsion and products at three different points. Mean and standard error for each parameter were calculated. Delta e (total colour difference) can be calculated by using following formula: -

$$\Delta e = \sqrt{(l^*_2 - l^*_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2}$$

3.3.2.4 Storage study

3.3.2.4.1 Thiobarbituric Acid Reactive Substances (TBARS) Number

The extraction method described by Witte et al. (1970) was used with suitable modifications for the determination of TBARS value in chevon patties. 5 gm of sample was triturated with 25 ml of pre cooled 20% trichloroacetic acid (TCA) in 2 M orthophosphoric acid solution for 2 min. The content was then quantitatively transferred into a beaker by rinsing with 25 ml of cold distilled water, mixed properly and filtered through ash less filter paper (Whatman filter paper No. 1, S. D. Fine Chemicals Ltd., Mumbai, India). Then 3 ml of TCA extract (filtrate) was mixed with 3 ml of TBA reagent (0.005 M) in test tubes and placed in a dark room for 16 h. A blank sample was made by mixing 1.5 ml of 20% TCA, 1.5 ml distilled water and 3 ml of 0.005 M TBA reagent. Absorbance (O.D.) was measured at fixed wavelength of 532 nm with a scanning range of 531 nm to 533 nm using UV-VIS spectrophotometer (Elico SL-159, Mumbai, India). TBARS number was calculated as mg malonaldehyde per kg of sample by multiplying O.D. value with a factor 5.2.

3.3.2.4.2 Free Fatty Acids

The free fatty acids (FFA) in the sample were quantified using method as described by Koniecko (1979). 5 gm of the chevon patties was blended for 2 min. with 30 ml of chloroform in the presence of anhydrous sodium sulphate. Then, it was filtered through Whatman filter paper No. 1 into a 250 ml conical flask. About 2 or 3 drops of 0.2 percent phenolphthalein indicator solution were added to the chloroform extract and titrated against 0.1N alcoholic potassium hydroxide with regular shaking till the end point, permanent pink colour appeared. The quantity of potassium hydroxide consumed during titration was recorded. Percent free fatty acid content was calculated as follows:

$$\text{Free fatty acid (FFA) (\%)} = \frac{0.1 \times \text{mL 0.1 N alcoholic KOH} \times 0.282}{\text{Sample weight (g)}} \times 100$$

3.3.2.4.3 Peroxide Value

The procedure as described by Koniecko (1979) was used with slight modifications. Five gram of chevon patties was blended for 2 min with 30 ml chloroform in the presence of anhydrous sodium sulphate. The mixture was filtered through Whatman filter paper No.1 and 25 ml aliquot of the filtered chloroform extract was transferred to 250 ml conical flask to which 30 ml of glacial acetic acid and 2 ml of saturated potassium iodide solution were added and allowed to stand for 2 min with occasional shaking (swirling). Thereafter 100 ml of distilled water and 2 ml of fresh 1 percent starch solution were added into the solution. Flask contents were titrated immediately against 0.1N sodium thiosulphate till the end point was reached (non-aqueous layer turned to colourless). The peroxide value (PV) was calculated in meq/kg of the meat as per the following formula:

$$\text{PV (meq/kg sample)} = \frac{0.1 \times \text{ml 0.1N sodium thiosulphate}}{\text{Sample weight (g)}} \times 1000$$

3.3.2.4.4 Microbiological Analysis

Standard Plate Counts, Total Coliforms count and psychrophilic count of the samples were enumerated following the methods as described by American Public Health Association (1984).

3.3.2.4.5 Preparation of Sample and Serial Dilutions

The samples were opened in an inoculation chamber of laminar flow (RH-58-03, Rescholar equipments, Ambala) pre-sterilized by ultra-violet (UV) radiation. 10 gm of sample from this was aseptically weighed and transferred to pre-sterilized mortar containing 90 ml of sterile 0.1% peptone water (RM001; Hi-Media Laboratories Pvt. Ltd., Mumbai). The sample was homogenized for 2 min using a sterile pestle and mortar for uniform dispersion and to get a 10^{-1} dilution of the sample. To prepare 10^{-2} dilution, 1 ml of this diluted solution was quantitatively transferred and then mixed uniformly in a test tube containing 9 ml of sterile 0.1% peptone water. Again 1 ml of 10^{-2} dilution was added to 9 ml 0.1% sterile peptone water and mixed to obtain 10^{-3} dilution and so on. Preparations of sample and serial dilutions were done near flame in a horizontal laminar flow apparatus observing all possible aseptic conditions. Serial dilutions were made as per requirement.

3.3.2.4.6 Standard Plate Count (SPC)

23.5 g of plate count agar (M091; Hi-Media Laboratories Pvt. Ltd., Mumbai) was suspended in 1000 ml glass of distilled water followed by boiling to dissolve the media completely and sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 min. Final pH of the medium was set at 7.0±0.2 at 25ºC. Pour plate technique was used. The plates were incubated at 35ºC for 48-72 hrs in an inverted position. Plates showing 30 to 300 colonies were counted manually. The average number of colonies was multiplied by reciprocal of the dilution and expressed as $\log_{10}$ cfu /g of sample.

3.3.2.4.7 Psychrophilic Counts

The same procedure as for SPC was followed for media preparation and plating. The plates were incubated at 4±1ºC for 10-14 days and the average number of colonies were multiplied by the reciprocal of the dilution and expressed as $\log_{10}$cfu/g of sample.

3.3.2.4.8 Coliform Counts

Coliform counts were enumerated using selective media; Violet Red Bile Agar (VRBA, M581A) procured from Hi- Media Laboratories Pvt. Ltd. Mumbai. A total of 51.53 g of media was suspended in 1000ml of distilled water, boiled to dissolve the medium completely and cooled to 45^{o}C. The final pH of the medium was adjusted to 7.4±0.2. One ml in duplicate of suitable dilution was pipetted into the sterilized

petridish. About 20 ml of the melted medium was poured over it, mixed slowly with rotating actions. The plates were allowed to stand for some time till the agar media got solidified. After solidification 4-5 ml of additional agar was added to form anaerobic layer and agar media was allowed to solidify. The plates were incubated at 35±2°C for 24 hrs. The numbers of red purple colonies with about 0.5 mm diameter surrounded by a zone of precipitated bile were counted. Colonies judged to be borderline cases were also counted. The average number of colonies was multiplied by the reciprocal of the dilution and expressed as $\log_{10}$ cfu/g.

3.3.2.5 Sensory Evaluation

A seven-member experienced panel of judges consisting of teachers and postgraduate students of department of LPT, of College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University evaluated the samples for the sensory attributes viz. colour and appearance, flavour, juiciness, texture and overall acceptability using 8-point descriptive scale Keeton (1983), where 8=excellent and 1=extremely poor. The test samples were presented to the panellists after assigning the suitable codes. The samples were warmed in a microwave oven for 20 sec before serving to the sensory panellists. The water was served for rinsing the mouth between the samples.

3.3.2.6 Statistical analysis

Data was analyzed statistically on 'SPSS-16.0' (SPSS Inc., Chicago, II USA) software package as per standard methods (Snedecor and Cochran 1994). Duplicate samples were drawn for each parameter and the whole set of experiment was repeated three times to have total six number of observations (n=6). Sensory evaluation was performed by a panel of seven member judges and experiment was repeated thrice, so total of 21 observations (n =21). The mean values were reported along with standard error. The statistical significance was estimated at 5% level ($p<0.05$) and evaluated with Duncan's Multiple Range Test (DMRT).

CHAPTER - IV

RESULTS AND DISCUSSION

In the present study, encapsulation of blended rosemary and oregano essential oils was carried out by spray drying and it was incorporated in chevon patties with an aim to extend its storage life. The levels of blending of essential oils to be used were already decided on the basis of *in-vitro* antimicrobial and antioxidant efficacy through series of experiments conducted in the lab. Encapsulation through spray drying was done at different inlet temperatures using maltodextrin and Gum Arabic at three concentrations viz. 2:1, 1:2 and 1:1. Incorporation of encapsulated powder was done in goat meat emulsion and evaluated at every 7 days of storage quality for 5 weeks. The above mentioned work was carried out through two different experiments and the present chapter concerns with results obtained through those experiments supported with statistically analysed tables and figures and discussed with relevant available findings.

4.1 Experiment No.1: Standardization of process protocols for the development of encapsulated blended Rosemary and oregano essential oil by spray drying and evaluation of its quality

The levels of blending of Rosemary (REO) and Oregano essential oil (OEO) was selected on the basis of Fractional Inhibitory Concentration Index (FICI) against common spoilage and pathogenic organisms and antioxidant activity on the basis of Radical Scavenging activity (ABTS and DPPH), earlier carried out in the department (Kaur, 2020) . Maltodextrin and Gum Arabic were used as wall materials for the experiment in three different combinations viz. 2:1 Maltodextrin: Gum Arabic, 1:2 Maltodextrin: Gum Arabic and 1:1 Maltodextrin: Gum Arabic. On the basis of preliminary trials and available literature, three different combinations of inlet temperatures that are 150°C, 170°C and 190°C were tried during spray drying. A total of 9 different combination (1: 1 MD:GA, 2:1 MD:GA, 1:2 MD:GA @ 150 ^{o}C, 170 ^{o}C, 190 ^{o}C each respectively) of biopolymer concentrations and inlet temperatures were tried for development of blended Rosemary and Oregano essential oil powder. The best level of encapsulated oil powder from biopolymer and inlet temperature combination was selected on the basis of higher encapsulation efficiency (EE), lower residual moisture percentage, shorter wettability time (sec), higher water solubility

(%) and bulk density and Tapped density. The selected combination was utilized in further experiments.

4.1.1. Encapsulation Efficiency (%) of spray-dried blended essential oils by using Maltodextrin – Gum Arabic combinations as wall material at three different inlet temperatures.

Results of Encapsulation Efficiency (%) of blended Rosemary and oregano essential oils obtained by using wall materials viz. Maltodextrin – Gum Arabic combinations (2:1 MD: GA), (1:2 MD: GA) and (1:1 MD: GA) at three different spray drying inlet temperatures viz. 150°C, 170°C and 190°C as presented in the Figure 1.

Encapsulation efficiency (EE) is the most significant quality parameter for encapsulation of any essential oil. It denotes the percentage of the initial amount of total essential oil that is encapsulated. A number of factors play an important role in determining EE viz. nature of wall material, concentration and drying temperature. In the present study, maltodextrin and gum Arabic at 1:1 ratio (MD: GA 1:1) at 190 °C inlet temperature combination showed lowest encapsulation efficiency (52.64 %) whereas, it was 57.67% and 54.24% at 1:2 and 2:1 MD: GA combinations, respectively at the same inlet temperature. The influence of high emulsifying temperature (>190 °C) on the emulsion viscosity is simply a physical effect as viscosity decreases with a corresponding rise in temperature. Moreover, high emulsifying temperatures (190 °C and more) might disrupt the affinity of the wall materials to each other and result in lower emulsion stability. In addition, the particles at a high air inlet temperature (190 °C) might be subjected to excessive evaporation which may result in cracks in the surface membrane promoting further leaching of oil from the particles leading to lower encapsulation efficiency. Similar behaviour was observed by Aghbashlo et al. (2013).

Highest encapsulation efficiency (%) was observed in maltodextrin and gum Arabic at 2:1 ratio (MD: GA 2:1) and 170 °C inlet temperature combination. It was observed that EE (%) significantly increased ($P<0.05$) with an increase in inlet temperature during spray drying from 150 °C to 170 °C, irrespective of the combination of wall material is used in it. However, at highest temperature (190 °C), there was lower values of EE (%).

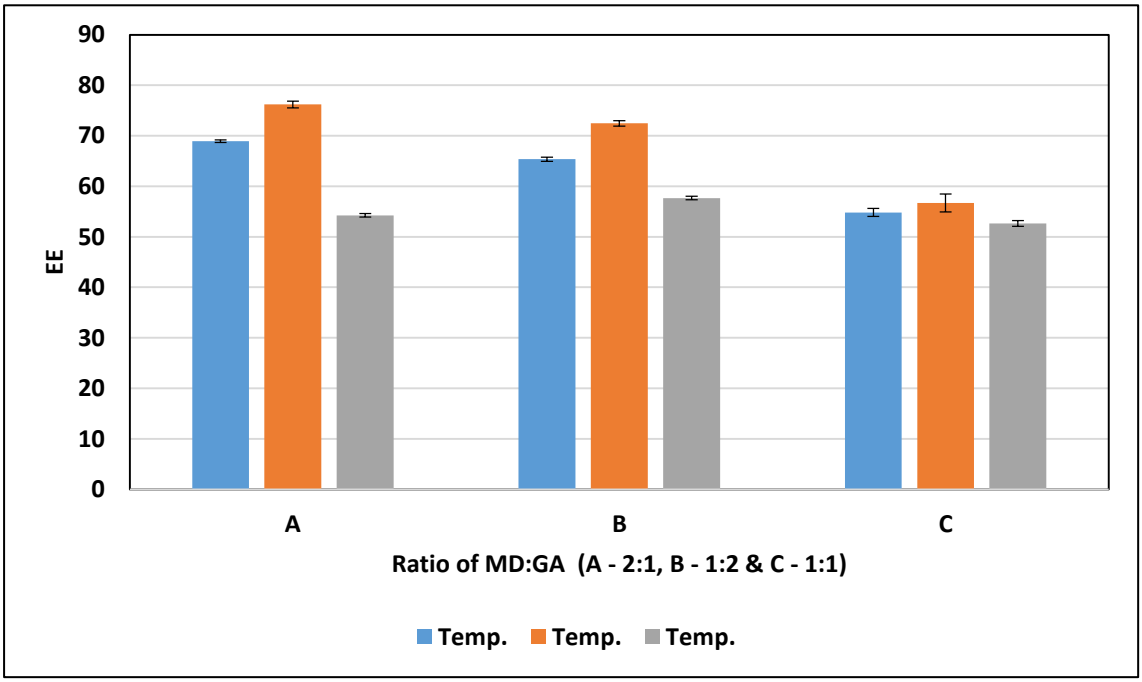


Fig 1. Effect of different wall material combinations and inlet temperature on encapsulation efficiency of blended essential oils during spray drying

A suitable viscosity and high emulsion stability could increase encapsulation efficiency and yield. Aghbashlo et al. (2012) found that as drying air temperature increased, the efficiency of fish oil encapsulation increased. Higher drying air temperatures accelerated the drying rate of droplets, promoting the fast formation of particle crust. The crust, as soon as formed, provided a firm membrane around the particles, preventing further leaching of oil from the droplet. The same behaviour was observed by Bhandari et al. (1992) in the encapsulation of citral and linalyl acetate by spray drying. Liu et al. (2000) also verified the positive effect of higher drying air temperature for retention of flavour during spray drying. The encapsulation efficiency and yield significantly increased as the air inlet temperature increased from 150°C to 170 °C, and then dropped due to breakage in crust of particles and subsequent leaching of core.

4.1.2 Wettability (s) of spray-dried blended essential oils by using Maltodextrin – Gum Arabic combinations as wall material at three different inlet temperatures.

Wettability is defined as the attraction of a liquid phase to a solid surface, and it is typically quantified using a contact angle with the solid phase. The wettability, or

ability to absorb water, from microcapsules is one of the most important physical properties related to reconstituting the powders (Bae and Lee 2008), and it is directly affected by the molecular interactions between the two phases Cuq B. et al., (2011). In other words it may be defined as the ability the spray dried powder to capture moisture or to hydrate. The wettability parameter explains the reconstituting power of the spray dried powder which is important physical property during the preparation of product with the incorporation of such powders.

In the present study, results of wettability for encapsulated powder using three different concentrations of maltodextrin and Gum Arabic combinations (2:1, 1:2 and 1:1) at three different inlet temperatures (150°C, 170°C, 190°C) are presented in the figure 2. In general, a significant increase ($p<0.05$) in inlet temperature of spray drier resulted in greater wettability time for encapsulated powders, irrespective of type and level of wall materials used. This could be due to rapid evaporation that further leads to production of powder with less moisture and more porosity. The findings are in consonance with that of Sanchez-Reinoso et al. (2017) & de Barros Fernandes et al. (2014a) who reported that an increase in drying temperature leads to greater reconstitution power for powders.

It was observed that the type of wall material had a significant ($P<0.05$) effect on the wettability time and was reported highest for powders with more proportion of gum Arabic than one with having more amount of maltodextrin as wall material. Perusal to figure 2, it was observed that 1:2 MD: GA combination showed the highest wettability time (s) as compared to 2:1 and 1:1 MD: GA powder combinations. The highest time taken to reconstitute by 1:2 MD: GA combination might be the due presence of a relatively higher concentration of Gum Arabic. This nature of Gum Arabic might be attributed to the hydrophobic and hydrophilic properties present in its molecular structure, namely arabinogalactan-protein fraction (AGP), which contains both hydrophobic polypeptide chains and hydrophilic polysaccharide blocks which provide it with excellent interfacial properties (Hu et al., 2019). Irrespective of inlet temperature, MD: GA 2:1 combination showed lowest wettability time as compared to other combinations. It might be due to the presence of a higher percentage of Maltodextrin as a wall material which is relatively faster to be reconstituted in water due to the presence of hydrophilic hydroxyls compounds in its composition. Souza et

al. (2018) & Edris A. E. et al. (2016). The higher percentage of moisture in any powder leads to agglomeration / caking which enables the liquid to penetrate into pores more easily further reducing the reconstitution time. Similar findings were observed by Fernandes et al. (2014) Ghosal et al. (2010) & Bae et al. (2008).

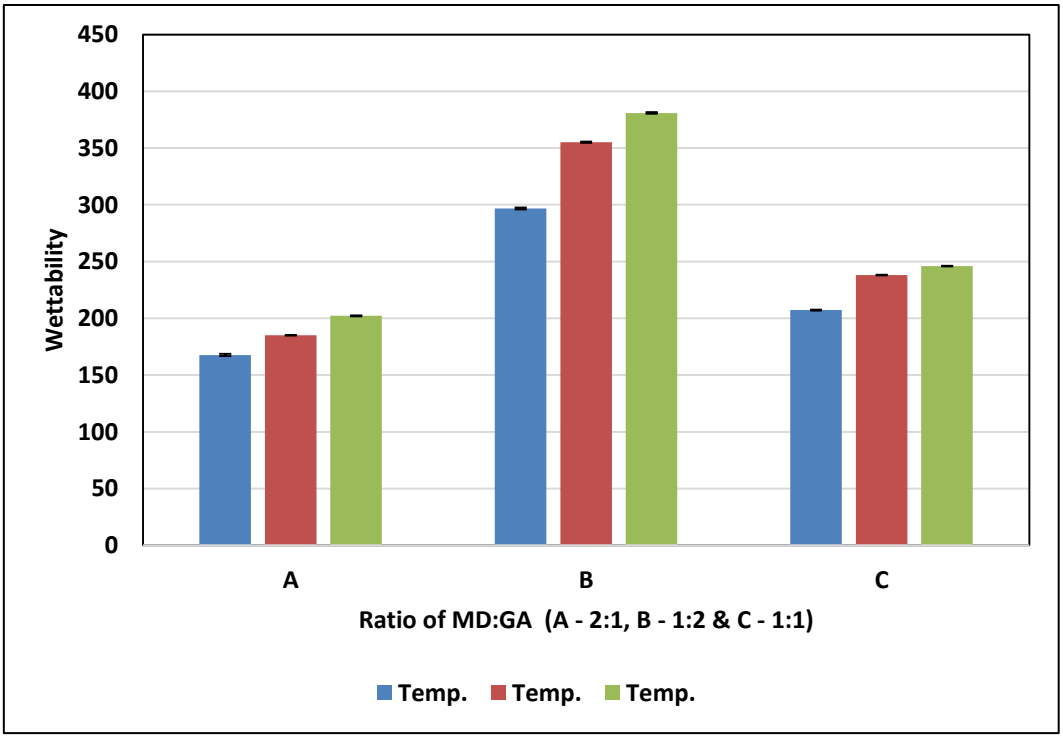


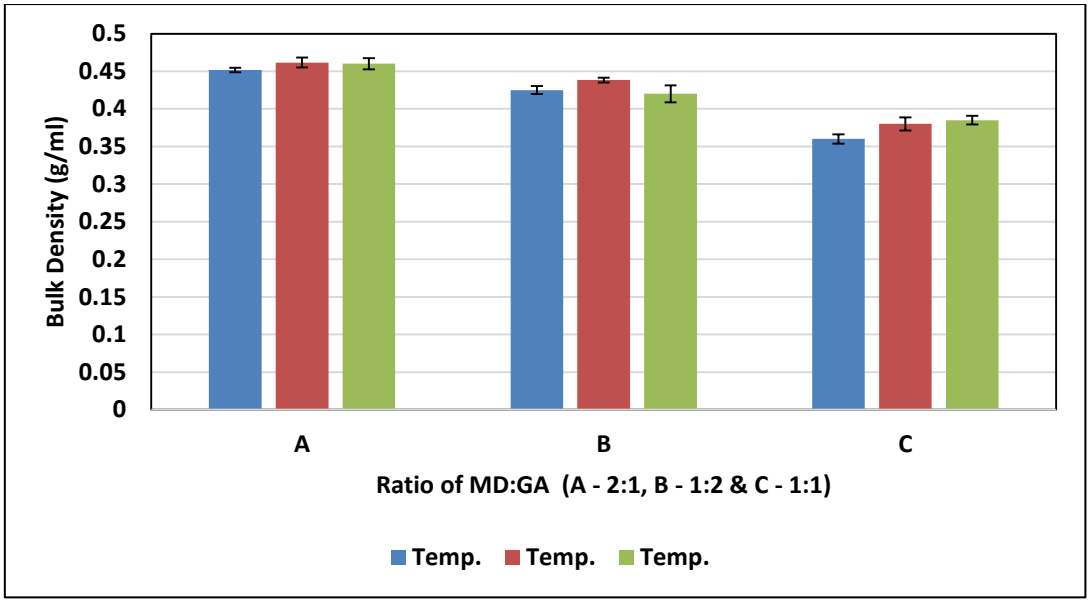
Fig 2: Effect of different wall material combinations and inlet temperature on wettability (%) of blended essential oils during spray drying

4.1.3. Bulk and tapped Density (gm/cm^3) of spray-dried blended essential oils by using Maltodextrin – Gum Arabic combinations as wall material at three different inlet temperatures.

The bulk density of a powder is the ratio of the mass of an untapped powder sample and its volume including the contribution of inter particulate void volume. Hence, the bulk density depends on both the density of powder particles and the spatial arrangement of particles in the powder bed. Higher the bulk density of a powder, greater is the stability due to the absence of consolidation of air inside the powder. It is an important property in the packaging, reconstitution, and retail disbursement of any powder. It is a factor controlled by size, shape, and moisture content (Shamei et al., 2017). In the present study, the effect of two different wall materials (Maltodextrin and Gum Arabic) at three different inlet temperatures was

studied. It was observed that type of wall material has a significant ($P<0.05$) impact on bulk density and it was noticed highest for MD: GA 2:1 combination, irrespective of temperature range. This might be because of the distinction in their residual moisture content. Papadakis et al. (1998) also have reported that the bulk density depends upon the residual moisture content of powders. The bulk density for 1:2 MD: GA combination ranges from 0.42 to 0.44 gm/cm^{3}at all the three different inlet temperatures and for MD: GA 1:1 combination, it ranged from 0.36 to 0.38 gm/cm^3 at the studied temperatures. The results are in concurrence with that of Raigar and Mishra (2015) who revealed an expansion in the bulk density with an increase in moisture content of the powder. Higher moisture content outcomes in clustering of particles, consequently increasing bulk density. With an increment in wall material concentration, bulk density of encapsulated powders ($P<0.05$) expanded. Similar findings have been reported by Chew et al. (2018) and Sanchez-Reinoso et al. (2017). This could be due to an increased moisture content of powder, irrespective of wall material. It was observed that the bulk density of powder decreased with increasing drying temperature. The effect of increasing inlet temperature on bulk density is depicted in Figure 3. This could be because of the creation of powders with lower residual moisture content at higher drying temperatures and further at a higher drying temperature empty microcapsules with higher sphericity and huge size particles will be framed that drives the occupation of more space. The findings were in consonance with Sanchez-Reinoso et al. (2017) & Shamaei et al. (2017).

Tapped density is an indispensable factor related to packaging, transport, and commercialization of powders; thus, this value can be useful in terms of weight and amount of material that will fit into a container (Botrel et al., 2012). Quispe-condori et al. (2011) have reported that a high density dry product can be stored in smaller containers in comparison with a low density product. On the similar lines as that of bulk density, type of wall materials affected tapped density too. This could be due to difference in the residual moisture content of the powders. It was observed that tapped density of powder decreased with increasing drying temperature. This could be due to production of powders with minimal moisture contents at higher inlet temperatures.



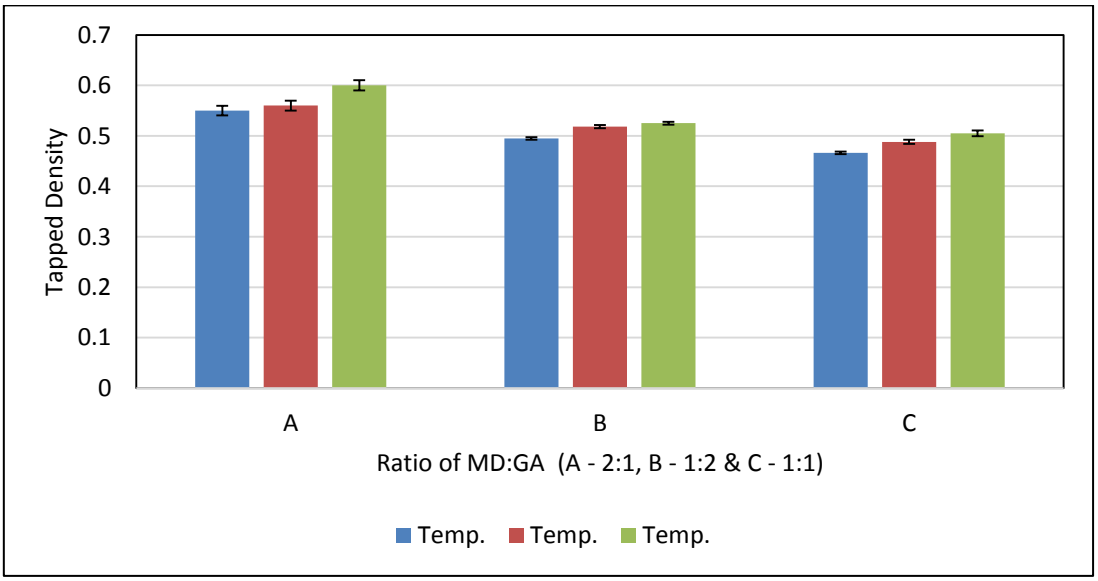


Fig 3. Effect of different wall material combinations and inlet temperature on density (g/ml) of blended essential oils during spray drying

4.1.4. Solubility (%) of spray-dried blended essential oils by using Maltodextrin – Gum Arabic combinations as wall material at three different inlet temperatures.

Solubility is the property of a solid, liquid, or a gaseous chemical substance called solute to dissolve in a solid, liquid, or gaseous solvent. It is one of the prominent reconstitution properties of powder. It refers to an ability of powder to dissolve in a given solvent. It also denotes the quality aspect of powder, which is necessary for its long shelf life and stability. Poorly soluble powders may cause processing difficulties and economic losses (Chew et al., 2018). The solubility of a substance depends on the temperature, pressure, and presence of other chemicals. It

also depends on the physical and chemical properties of the solute and solvent. It was observed that the type of wall material used had a significant ($P<0.05$) effect on the solubility. Among three combinations, MD: GA 2:1 combination showed the highest solubility percentage of 70.18 % at 190°C temperature. It might be due to the low viscosity and the more soluble nature of maltodextrin when mixed with water. Souza et al. (2016) & Lacerda et al. (2017) reported similar results using maltodextrin as a wall material during encapsulation. On the other hand, the combination having relatively higher percentage of gum Arabic as wall material showed lowest solubility irrespective of inlet temperature applied. Lower solubility might be due to higher percentages of Gum Arabic in the combination which has a higher water holding capacity than maltodextrin that further promotes crystal formation at the bottom and results in less solubility of powders. It was observed that an increase in the inlet temperatures from 150°C to 190°C significantly ($P<0.05$) increased solubility percentages of spray-dried powders irrespective of type and level of wall materials used. It might be due to the rapid evaporation of moisture from wall materials and making them more porous in nature. Further at higher inlet temperature there is production of larger particles which ultimately enhance solubility of powder. In the present study, the solubility percentages ranged from 52.80% to 70.81%. The findings are in consonance with that of Sanchez-Reinoso et al. (2017) who reported increase in inlet temperature increases solubility of powders.

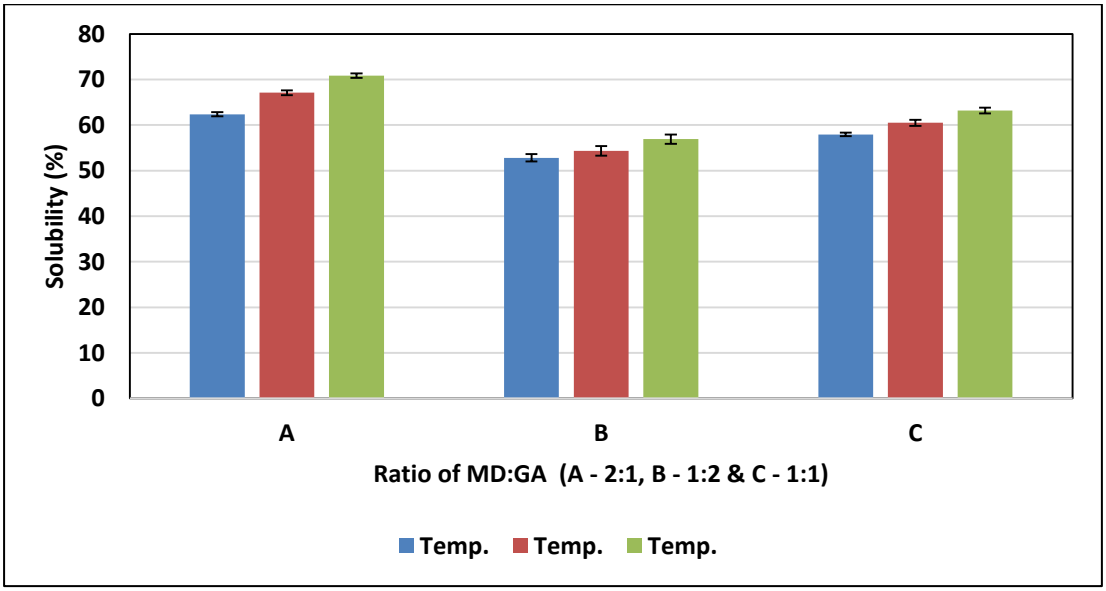


Fig 4: Effect of different wall material combinations and inlet temperature on solubility (%) of blended essential oils during spray drying

4.1.5. Moisture content (%) of spray-dried blended essential oils by using Maltodextrin – Gum Arabic combinations as wall material at three different inlet temperatures.

The residual the moisture content of spray-dried powder is a significant attribute for analysing its stability, hygroscopicity, bulk density, acceptability, and flowability (Chew et al., 2018). The moisture content of more than 4% in spray-dried powder is not desirable, because increased moisture percentage fastens the process of oxidation and increases the risk of microbial contamination (Sanatana et al., 2016). Inlet temperatures during the spray drying process is one of the most important factors which plays a major role in deciding the moisture content of the powder. In the present study, significant decrease ($P<0.05$) in the residual moisture content (%) with an increase in inlet temperature from 150°C to 190°C range, irrespective of type and combination of the wall the material used for spray drying, was observed.

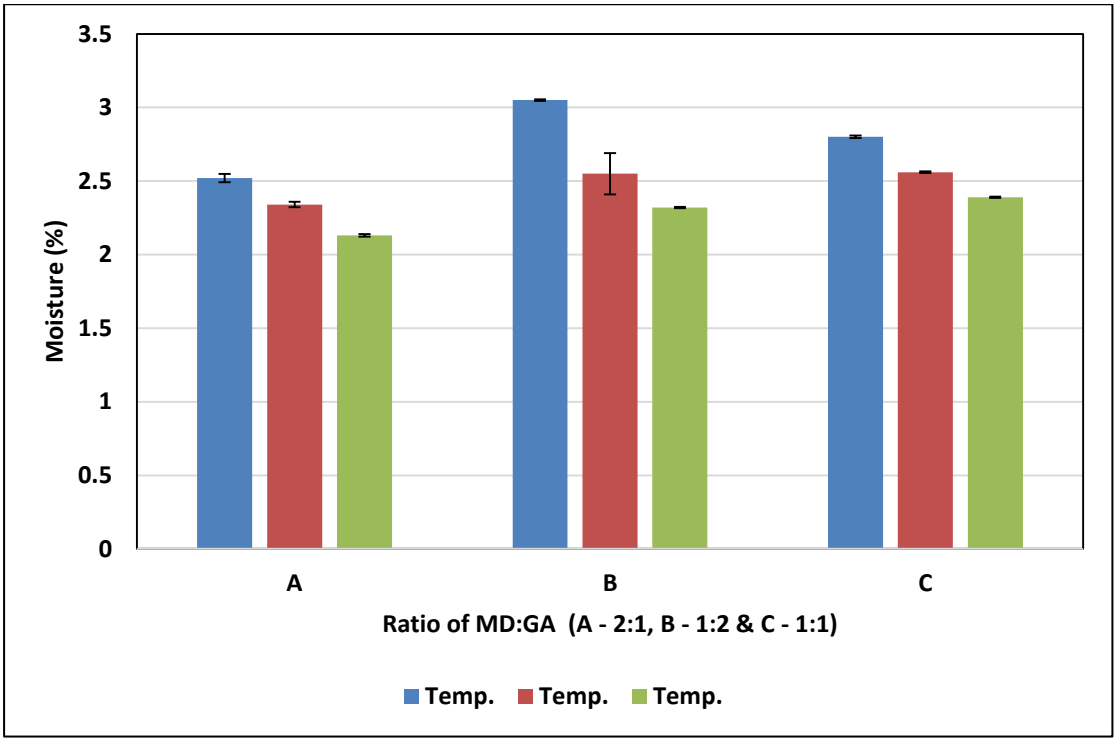


Fig 5: Effect of different wall material combinations and inlet temperature on moisture (%) of blended essential oils during spray drying

This could be due to temperature difference between particles and drying medium. The greater difference would lead to high rate of heat transfer, subsequently resulting in lower moisture content. The residual moisture content (%) obtained after spray drying of blended rosemary and oregano essential oil with three different

combinations of wall materials and inlet temperature combinations are represented figure 5. Out of all wall materials combinations used, MD: GA 1:2 combination showed highest residual moisture percentage. The type of wall material ultimately decides the emulsion characteristics such as stability, viscosity, particle size as well as different powder properties such as surface oil density, morphology, and oxidative stability (Jafari et al., 2008). The lower residual moisture content in powder with higher amount of maltodextrin as wall material could be due to its lower emulsion viscosity. Gum Arabic is reported to have viscous emulsion characteristics due to its thickening property and a ramified structure with long chains (Carneiro et al., 2013), which helps in having more residual moisture in that group.

Out of all the treatments, based on Encapsulation efficiency, bulk density, tapped density, solubility and wettability; combination of maltodextrin and gum Arabic at 2:1 ratio as wall material, at 18% concentration and 170°C inlet temperature was selected as spray drying conditions and parameters for encapsulation of blended rosemary and oregano essential oil.

4.2 Experiment No.2: To optimize the incorporation levels of encapsulated blended essential oils in chevon patties and evaluation of its storage stability at refrigeration temperature (4 ±1ºC).

4.2.1 Experiment No. 2 (a): Optimization of the incorporation levels of encapsulated powder in goat meat emulsion

Three different levels viz. 0.5%, 1% and 1.5% of encapsulated blended essential oil powders (Experiment No. 1) were incorporated separately in goat meat emulsion, replacing the lean meat in the pre-standardized formulation. The goat meat emulsion incorporated with three different levels of encapsulated powder was analyzed for physico-chemical properties (emulsion pH and stability), sensory evaluation (Appearance, flavour, texture, juiciness and overall acceptability) and instrumental colour profile.

4.2.1.1 Physico-chemical, sensory and instrumental colour analysis of goat meat emulsion incorporated with encapsulated blended essential oil powder

The results for Physico-chemical properties (emulsion pH and stability), sensory evaluation (Appearance, flavour, texture, juiciness and overall acceptability)

and instrumental colour profile of goat meat emulsion incorporated with the three different levels of encapsulated powder are presented in Table 4.

4.2.1.1.1 Physico-chemical characteristics

The pH of goat meat emulsion decreased significantly ($P<0.05$) with incorporation of encapsulated powder as compared to control. Lower pH value was observed for T_3 and this could be due to lower pH value of blended essential oils as compared to chevon. An increase ($P<0.05$) in emulsion stability was observed with incorporation of encapsulated powders due to higher emulsification capacity of added encapsulated powders, due to presence of wall materials which act as emulsifiers and stabilizers in meat matrix. Similar increase in emulsion stability has reported by Candogan and Kolsarici (2003) by addition of carrageenan in low-fat beef frankfurters.

4.2.1.1.2 Sensory analysis

On incorporation of different levels of encapsulated powders in goat meat emulsion, a change in sensory scores was observed, which is presented in table 4. The sensory scores for appearance in all the treatment products were significantly lower ($P<0.05$) than control. It could be due to replacement of chevon with blended essential oil powder that resulted in dilution of bright colour of meat product. The flavour scores for all treated products was lower than control due to distinct flavour attribute of essential oils, however, the scores for T_1 and T_2 were comparable. Flavour scores of T_3 were significantly lower ($P<0.05$) than control and other treatments. The sensory scores for texture and juiciness increased significantly ($P<0.05$) in treatments as compared to control which might be due to an increase in water holding capacity and good binding ability because of presence of wall materials such as gum arabic and maltodextrin in the treated products. Similar findings have also been reported by Garcia et al. (2013) in charqui with addition of carrageenan. The overall acceptability scores for all the treatments products was significantly lower ($P<0.05$) than control which is in correlation with the scores of other sensory parameters. However, the scores for T_1 and T_2 were comparable to each other and higher than T_3.

Table 4: Effect of incorporation of different levels of encapsulated spray dried powder on Physico-chemical, Sensory and instrumental colour profile of goat meat emulsion (Mean±S.E.)

Sr. No.	Parameters	Control	Encapsulated powder levels		
			T_1 (0.5%)	T_2 (1%)	T_3 (1.5%)
Physico-chemical properties					
1.	Emulsion pH	6.64±0.02^{a}	6.48±0.02^{b}	6.40±0.01^{c}	6.32±0.01^{c}
2.	Emulsion Stability	84.38±0.41^{d}	85.25±0.12^{c}	86.00±0.27^{b}	87.29±0.24^{a}
Sensory Analysis					
3.	Appearance	7.30±0.03^{a}	7.20±0.04^{b}	7.14±0.06^{b}	6.85±0.06^{c}
4.	Flavour	7.20±0.04^{a}	6.83±0.04^{b}	6.72±0.05^{b}	5.90±0.05^{c}
5.	Texture	7.00±0.05^{c}	7.11±0.02^{b}	7.21±0.04^{b}	7.32±0.02^{a}
6.	Juiciness	7.04±0.04^{b}	7.10±0.02^{a}	7.10±0.04^{a}	7.12±0.03^{a}
7.	Overall Acceptability	7.11±0.03^{a}	7.02±0.03^{b}	6.95±0.05^{b}	6.00±0.04^{c}
Instrumental colour Profile					
8.	Redness (a* value)	13.36±0.27^{a}	12.14±0.36^{b}	12.21±0.22^{b}	09.38±0.18^{c}
9.	Yellowness (b* value)	16.66±0.40	17.23±0.61	17.54±0.91	17.73±0.72
10.	Lightness (l*)	41.22±0.83^{c}	46.2±0.48^{bc}	47.35±0.60^{ab}	48.70±0.35^{a}

N=6, *Mean±S.E. with different superscripts row wise (a-d) differ significantly ($p< 0.05$).

4.2.1.1.3 Instrumental colour profile

The value for redness decreased ($P<0.05$) with increasing level of incorporation of encapsulated powder in goat meat emulsion. This could be attributed to lighter colour of encapsulated powder that might have diluted the desirable bright red colour of goat meat emulsion. The findings are in congruence with that of sensory scores for appearance. The value for lightness also showed trend corresponding to decrease in redness values of emulsion.

On the basis of Physico-chemical, sensory and instrumental colour analysis, goat meat emulsion incorporated with 1% encapsulated blended essential oil powder by replacing the lean meat in the standardized formulation was found to be most suitable for the development of chevon patties.

4.2.2 Experiment No. 2 (b): Evaluation of the storage stability of developed chevon patties incorporated with encapsulated essential oil powder at refrigeration temperature (4±1°C).

Chevon patties, both control and the one incorporated with selected level of (1%) encapsulated blended essential oil powder was developed and stored under aerobic packaging conditions at refrigeration temperature (4±1ºC). The effect of treatment provided and refrigerated storage (4 ±1ºC) on the storage quality of both control and treated products was assessed at regular intervals. Four different batches of products were developed viz. **C**-Control with aerobic packaging, **T_1**-Product with spray dried encapsulated rosemary essential oil (4.5%) powder, **T_2** - Product with spray dried encapsulated oregano essential oil (4.5%) powder, and **T_3**. -Product with spray dried Blended essential oil encapsulated powder. The samples were drawn at a regular interval of 7 days on 0, 7, 14, 21, 28 and 35 days. The storage quality was evaluated on the basis of Physico-chemical (pH, Thiobarbituric Acid Reactive Substances (TBARS), Free Fatty Acids content and Peroxide value), microbiological parameters and sensory. The statistically analyzed results are presented in Fig. 6 and 7 and discussed in the light of objectives.

4.2.2.1 Physico-chemical quality parameters

The results for the Physico-chemical parameters such as pH, TBARS value, FFA and peroxide value for chevon patties are presented in Fig. 6.

4.2.2.1.1 pH

For both control and treatments stored at refrigeration temperature, pH varied significantly ($P<0.05$) with the passage of storage interval. It showed an increasing trend throughout the storage period, irrespective of the treatment applied**.** It might be attributed to accumulation of alkaline compounds, such as ammonia mainly formed due to microbial action (Hadian et al., 2017 & Ozyurt et al., 2012). However, for treated products, the increase in pH was slower as compared to control and amongst the stored treatments, T_3 had lowest pH change at end of storage as compared other products due to protective effect of blended essential oil, which might have shown a sustained and sequential release during storage period.

4.2.2.1.2 Thiobarbituric acids reacting substances (TBARS) value

An indicative of lipid oxidation in meat products, TBARS value is a very important parameter. TBARS showed a significant ($P<0.05$) increasing trend throughout the storage interval for all the treatments as well as control. It could be possibly due to increased lipid oxidation and production of volatile metabolites during refrigerated storage. Similar findings had been observed for different meat products stored at refrigeration temperature by Mehta et al. (2016). At every storage interval, the value for TBARS was lower ($P<0.05$) than that of control product, which could be due to protective effect of encapsulated essential oils, however, lowest increase in value was observed in T_3. It could be due to synergistic effect of both oil blends as compared to the individual action. In general, presence of essential oils in the products ensured antioxidant action and retarded rate of lipid oxidation during storage of meat products. The findings are in consonance with Kahraman et al. (2015) who reported that poultry fillets to which REO added had significantly lower TBAR values ($p < 0.01$) than the untreated poultry fillets**.** Karoui and Hassoun (2017) have reported significantly lower TBARS value for Atlantic mackerel (*Scomber scombrus*) fillets treated with REO during storage period. A similar decrease in TBARS values on incorporation of rosemary extracts have been reported by Pereira and Pinhgeiro (2017) in chicken burgers during refrigerated storage(4±1°C). Selani et al. (2011) and Al-Kahtani et al. (1996) reported that meat products were considered to be fit for consumption if they had values below 1 mg of malonaldehyde/kg meat. According to these findings, chevon patties containing encapsulated powders were deemed fit for consumption after 35 days of storage and T_3 performed better than rest.

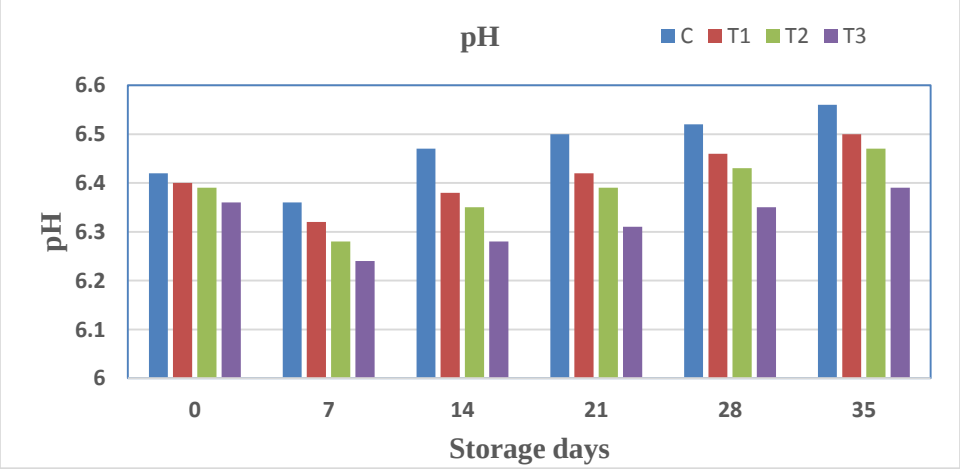
4.2.2.1.3 Free fatty acids (FFA)

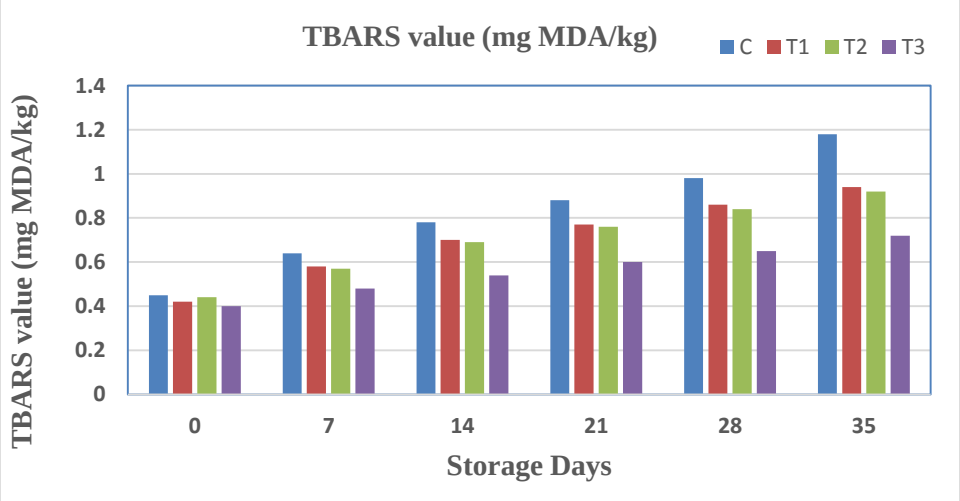
These are the products of enzymatic or microbiological degradation of lipids and indicator of fat stability during storage. In the present study, FFA values were comparable amongst all treatments and control on 0 day of storage. However, an increase ($P<0.05$) in FFA content during storage on the similar lines of TBARS had been observed, irrespective of treatments. The treated samples had significantly lower ($P<0.05$) free fatty acid content than control products during entire period of storage. Similar findings have been reported by Fernandes et al. (2018) & Kenar et al.(2010). Amongst the treatments, a significantly lower ($P<0.05$) FFA value was noticed for T_3 throughout the storage period which might be due to strong antioxidant activity of

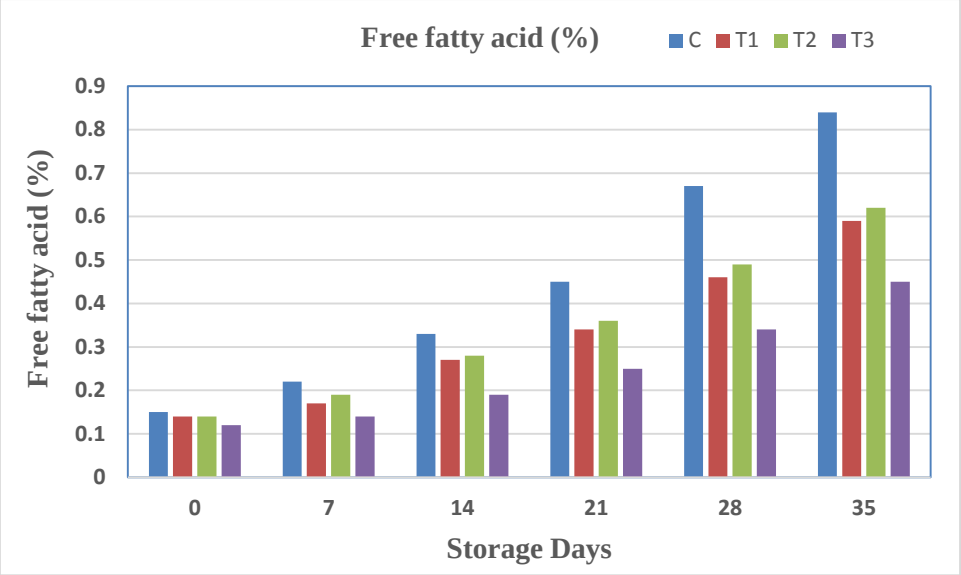
blended essential oil. Pearson (1968) reported that minced beef had FFA content in the range of 0.38 to 1.74% and had a maximum acceptability limit of 1.8% FFA in view of their progressive increase during storage. In the present study, FFA content of all the products was well below this limit.

4.2.2.1.4 Peroxide value

Both control and treated products followed the similar significantly increasing trend as that of TBARS and FFA values, and the value was significantly lower ($P<0.05$) in treated products throughout the storage period except on 0 day of storage. It could be due to inhibition of lipid peroxidation, attributed to encapsulated powders which function as antioxidants by terminating free radical chain-type reactions. The findings are in consonance with findings of Fernandes et al. (2018) and Gao et al. (2014).







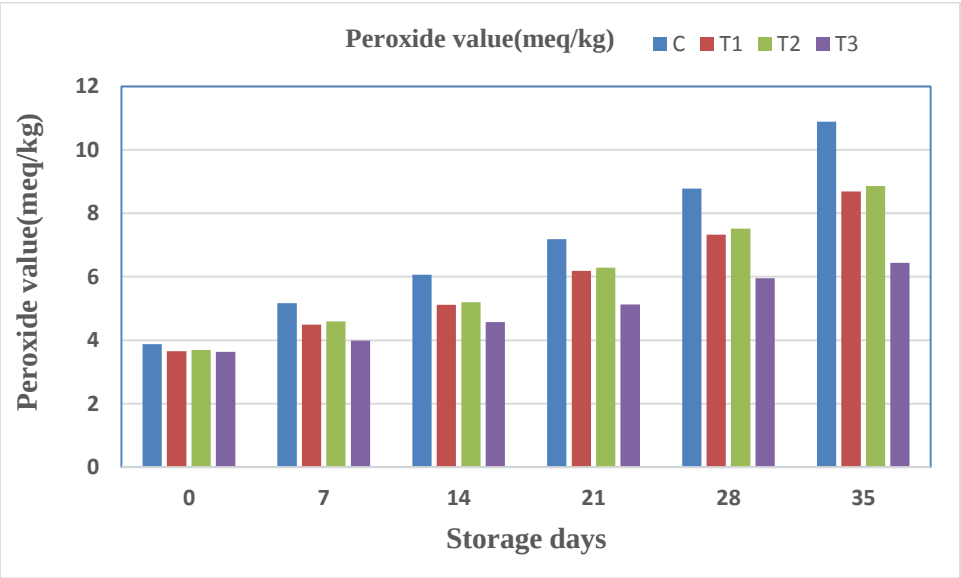


Fig. 6: Effect of incorporation of encapsulated essential oil powders on the physicochemical parameters of Chevon patties during storage at refrigeration temperature (4±1°C) (Mean± S.E.)* (n=6, C: control; T1: Product with spray dried encapsulated rosemary essential oil (4.5%) powder, T2: Product with spray dried encapsulated rosemary essential oil (4.5%) powder; T3: Product with spray dried Blended encapsulated powder.)

4.2.2.2 Microbiological quality

The microbiological quality parameters (standard plate count, psychrophilic count and coliform count) for the control as well treated chevon patties as detected on 0, 7, 14, 21, 28 and 35 days of refrigerated storage (4±1°C) under aerobic packaging are presented in Fig 7.

In the present study, on the initial day, no significant change in SPC values

between different products was observed, however the mean values of the standard plate count (SPC) significantly (P< 0.05) increased at each subsequent storage interval, irrespective of treatment given. A significant (P<0.05) influence of addition of encapsulated essential oil powders was observed at each day of storage. It could be due to sustained release of essential oil from encapsulated matrix resulting in prominent antimicrobial action. Similar findings have reported by various studies conducted by Rizzo et al. (2018). T_3 was reported to have least microbial load after 35 days storage as compared to other treated products. After 35 days storage, the values for SPC were well below the permissible limit of $log_{10}7$ cfu/g for cooked meat products Jay. (1996) in all the treatments except control. On 21st day of storage, psychrophiles were first detected in control and on 28th day of storage in T_1 and T_2. However, they were not reported in T_3 throughout the storage period. It could be due to synergistic inhibitory effect of blended essential oils. Similar findings have been reported by Dong et al .(2018) who found that the shrimp packaged in active films containing REO showed lower Psychrophilic count values compared to samples packed in control films during storage period of 10 days at 4 °C. In control as well as treatment products, the coliforms were not detected throughout the storage period of 35 days. It could be due to the thermal destruction of coliforms during cooking and hygienic practices followed during handling and packaging of chevon patties. Similar results were reported by Melo et al. (2012) with refrigerated chicken meat in contact with cellulose acetate-based film incorporated with rosemary essential oil.

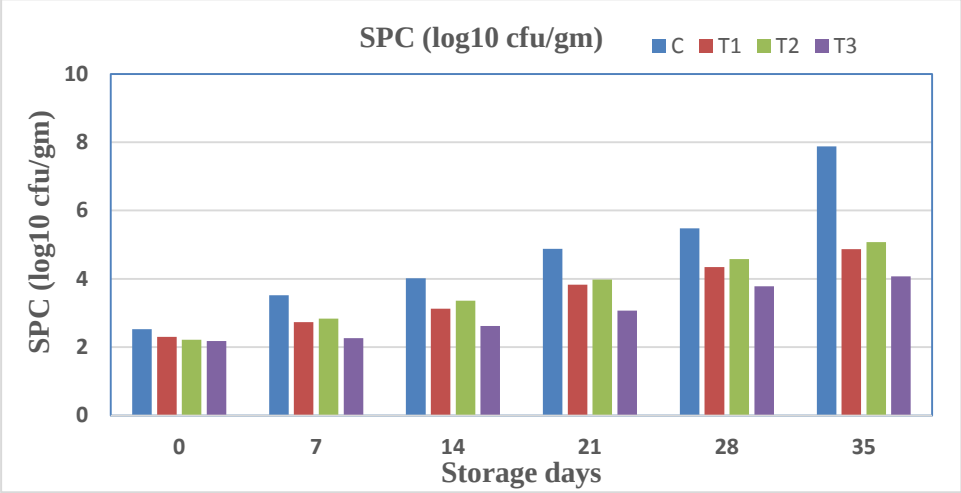


Fig 7: Effect of incorporation of encapsulated essential oil powders on the microbiological parameters of Chevon patties during storage at refrigeration temperature (4±1°C) (Mean± S.E.)*

4.2.3 Sensory analysis of chevon patties incorporated with encapsulated essential oil powder stored at refrigeration temperature (4±1°C) under aerobic packaging conditions

Mean sensory scores of chevon patties during storage at 4±1°C are presented in Table 5. A decreasing trend with a subsequent increase in storage interval was observed, irrespective of treatment applied. The general appearance scores for all the treatment products and control showed a progressive significant ($P< 0.05$) decline with increase in storage period but the rate of decrease in the scores for the treatment products was lower as compared to control. On 35^{th} day of storage, the control product was not evaluated for all the sensory parameters since the values of microbial counts were quite high for the same and started the appearance of sliminess. Out of all the products, T_3 showed the highest appearance score on 35^{th} day of storage which could be due to synergistic action of blended rosemary and oregano essential oils in encapsulated form. A significant decrease ($P<0.05$) in flavour scores for both control and treatments was observed. It could be due to increased lipid oxidation, liberation of fatty acids and increased microbial load as evident by lipid oxidation parameters and microbiological counts in study. However, it was much lower for treated products and lowest for T_3. A decreasing trend for Juiciness and texture scores observed throughout the storage, irrespective of treatment given and the rate of decrease was lower ($P< 0.05$) in treated product as compared to control products. A progressive decrease in texture and juiciness scores might due to proteolytic and lipolytic changes (Joukar et al., 2017). The texture and juiciness scores were significantly higher ($P<0.05$) in T_3 than other treatments and control on 35^{th}day of storage. For overall acceptability scores, decreasing trend in the both groups i.e. control and treatment was observed with the progression of storage period. At the end of the storage period T_3 was reported to have highest overall acceptability score i.e. 6.57.

Table 5: Effect of incorporation of encapsulated essential oil powders on the Sensory attributes of Chevon patties during storage at refrigeration temperature (4±1°C) (Mean± S.E.)*

Treatment	0 day	7 day	14 day	21 day	28 day	35 day
Colour/appearance						
C	7.13±0.07^{a}	6.92±0.04^{bC}	6.65±0.04^{cC}	6.37±0.04^{dC}	6.22±0.04^{eD}	NP
T_1	7.14±0.04^{a}	7.02±0.03^{bB}	6.90±0.03^{cB}	6.71±0.04^{dB}	6.56±0.04^{eB}	6.29±0.04^{fB}
T_2	7.18±0.02^{a}	7.11±0.03^{bA}	6.96±0.03^{cA}	6.77±0.03^{dB}	6.48±0.04^{eC}	6.10±0.03^{fC}
T_3	7.11±0.06^{a}	7.08±0.05^{bA}	6.98±0.03^{cA}	6.86±0.03^{dA}	6.71±0.03^{eA}	6.68±0.03^{eA}
Flavour						
C	7.44±0.03^{aA}	7.13±0.05^{b}	6.72±0.01^{cC}	6.50±0.03^{dC}	6.11±0.04^{eC}	NP
T_1	7.10±0.04^{aB}	7.06±0.03^{ab}	6.87±0.05^{bB}	6.65±0.03^{cB}	6.49±0.07^{dB}	6.33±0.03^{eC}
T_2	7.12±0.03^{aB}	7.10±0.03^{b}	6.89 ±0.05^{cB}	6.71±0.08^{dB}	6.55±0.07^{eB}	6.41±0.02^{fB}
T_3	7.16±0.04^{aB}	7.13±0.02^{b}	7.04±0.04^{bA}	6.90±0.03^{cA}	6.72±0.07^{dA}	6.63±0.03^{eA}
Texture						
C	7.13±0.04^{aB}	6.91±0.03^{bC}	6.65±0.02^{cC}	6.54±0.04^{dC}	6.30±0.04^{eC}	NP
T_1	7.28±0.01^{aA}	7.11±0.02^{bB}	6.90±0.07^{cB}	6.73±0.02^{dB}	6.50±0.03^{eB}	6.34±0.03^{fB}
T_2	7.21±0.01^{aA}	7.17±0.04^{bB}	6.93±0.02^{cB}	6.72±0.05^{dB}	6.54±0.03^{eB}	6.34±0.02^{fB}
T_3	7.23±0.06^{aA}	7.20±0.03^{bA}	7.05±0.03^{cA}	6.88±0.02^{dA}	6.72±0.05^{eA}	6.59±0.05^{fA}
Juiciness						
C	7.03±0.06^{aB}	6.80±0.04^{bC}	6.61±0.05^{cC}	6.50±0.02^{dC}	6.22±0.05^{eC}	NP
T_1	7.15±0.02^{aA}	6.91±0.04^{bB}	6.76±0.04^{cB}	6.67±0.04^{cB}	6.54±0.04^{dB}	6.23±0.04^{eC}
T_2	7.16±0.04^{aA}	7.00±0.03^{bB}	6.81±0.03^{cB}	6.73±0.05^{dB}	6.53±0.02^{eB}	6.32±0.03^{fB}
T_3	7.19±0.04^{aA}	7.07±0.04^{bA}	6.92±0.05^{cA}	6.88±0.04^{cA}	6.73±0.08^{dA}	6.60±0.02^{eA}
Overall acceptability						
C	7.20±0.06^{a}	7.00±0.03^{bB}	6.70±0.03^{cC}	6.32±0.08^{dC}	6.00±0.05^{eC}	NP
T_1	7.21±0.04^{a}	7.07±0.03^{bA}	6.80±0.03^{cB}	6.62±0.02^{dB}	6.40±0.03^{eB}	6.24±0.03^{fB}
T_2	7.15±0.04^{a}	7.07±0.04^{bA}	6.82±0.02^{cB}	6.58±0.04^{dB}	6.40±0.02^{eB}	6.19±0.04^{fB}
T_3	7.18±0.02^{a}	7.11±0.02^{bA}	6.91±0.05^{cA}	6.76±0.02^{dA}	6.62±0.04^{eA}	6.57±0.03^{eA}

n=21, C: control; T1: Product with spray dried encapsulated rosemary essential oil (4.5%) powder, T2: Product with spray dried encapsulated rosemary essential oil (4.5%) powder; T3: Product with spray dried Blended encapsulated powder

N.P.-Not Performed

**8-point descriptive scale, where 1-extremely undesirable and 8-extremely desirable.

* Mean±S.E. with different superscripts row wise (a-f) and column wise (A-C) differ significantly (P< 0.05).

Decrease in overall acceptability scores during storage might be due to corresponding decrease in other sensory parameters viz. appearance and colour, flavour, juiciness, and texture. Similar findings have been reported by a number of workers in various meat products stored at refrigeration temperature (4±1°C). Hence it can be concluded that the products incorporated with encapsulated essential oil powders can be stored better than control for 35 days under aerobic packaging conditions. However, the one incorporated with blended essential oil powders can be stored without any significant loss in Physico-chemical, microbiological and sensory properties under refrigerated storage.

CHAPTER - V

SUMMARY AND CONCLUSIONS

In last decade, there has been an incremental interest in research for the production of biologically active compounds from natural sources that can retard the oxidation of fat and control the microbial growth and multiplication in meat products. These protective abilities of bioactive materials are mostly attributed to plant polyphenols along with their antioxidant, antimicrobial and antiviral effects. Essential oils are volatile, natural, complex compounds, characterized by strong odour and are produced by aromatic plants as secondary metabolites. Their antimicrobial action is attributed to various phenolic compounds. However, direct incorporation of these antimicrobial agents in meat products result in immediate and short term reduction of bacterial populations due to destruction of active principle during processing. Also, it possesses strong flavour which results in poor organoleptic acceptability. These challenges can be overcome by encapsulating the essential oil in suitable matrix system that would ensure stability and progressive release of active agent without any detrimental effect on sensory attributes. The synergism can also be explored for better results. The objectives of the present study were achieved through carrying out two different experiments which are summarized and concluded in this chapter.

5.1 Experiment No.1: Standardization of process protocols for the development of encapsulated blended Rosemary and oregano essential oil by spray drying and evaluation of its quality.

The level of Rosemary and Oregano oil for the blending was selected on the basis of Fractional Inhibitory Concentration Index (FICI) and antioxidant activity of blended oils was done on the basis of radical scavenging activity (DPPH/ABTS) against common spoilage and pathogenic organisms. Maltodextrin and Gum Arabic were used as wall material for the experiment in three different combinations viz. 2:1 Maltodextrin: Gum Arabic, 1:2 Maltodextrin: Gum Arabic and 1:1 Maltodextrin: Gum Arabic. A total of 9 different combination of biopolymer concentration and inlet temperature was tried and developed blended Rosemary and Oregano essential oil powder. The best level of encapsulated oil powder from biopolymer and inlet temperature combination will be selected on the basis of higher encapsulation efficiency (EE), lower residual moisture percentage, shorter wettability time (sec),

higher water solubility (%) and bulk density and Tapped density. On the basis of evaluation of parameters, combination of MD and GA (2:1) as wall material at 18% concentration and 170°C inlet temperature had most favourable powder properties and used for further studies in the experiment.

5.2 Experiment No.2: To optimize the incorporation levels of encapsulated blended essential oils in chevon patties and evaluation of its storage stability at refrigeration temperature (4 ±1°C).

5.2.1 Experiment No. 2 (a): Optimization of the incorporation levels of encapsulated powder in goat meat emulsion

Three different levels viz. T_1 (0.5%), T_2 (1%) and T_3 (1.5%) encapsulated blended essential oil powders (Experiment No. 1) were incorporated separately in goat meat emulsion, replacing the lean meat in the pre-standardized formulation. The goat meat emulsion incorporated with three different levels of encapsulated powder was analysed for Physico-chemical properties (emulsion pH and stability), sensory evaluation (Appearance, flavour, texture, juiciness and overall acceptability) and instrumental colour profile. The pH of goat meat emulsion decreased significantly ($P<0.05$) with incorporation of powder as compared to control. It might be attributed to lower pH value of essential oils powder as compared to chevon. A significant ($P<0.05$) increase in emulsion stability with increasing levels was recorded, highest being in T_3 and lowest in control. The sensory scores for appearance for all the treatment products were significantly lower ($P<0.05$) than control which might be due to lighter colour of incorporated powder resulting in dilution of bright red colour of chevon emulsion. The flavor scores of T_3 were significantly lower ($P<0.05$) than control and other treatments. The texture and juiciness scores increased significantly ($P<0.05$) in treatments as compared to control that might be due to an increase in water holding capacity. The decreasing trend was noticed in redness value and increasing trend was noticed in lightness (L^*). On the basis of Physico-chemical, sensory and instrumental colour analysis, goat meat emulsion incorporated with 1% level of encapsulated blended essential oil powder, by replacing the lean meat in the standardized formulation was found to be most suitable for the development of chevon patties.

5.2.2 Experiment No. 2 (b): Evaluation of the storage stability of developed chevon patties incorporated with 1% blended essential oil encapsulated powder at refrigeration temperature (4±1°C).

Control and treated chevon patties from previous experiment incorporated with 1% blended essential oil powder were developed and stored under aerobic packaging conditions at refrigeration temperature (4±1ºC). The effect of refrigerated storage (4 ±1ºC) on the quality of the control and treated products was assessed. In total, four different products viz. C-Control with aerobic packaging, T_1-Product with spray dried encapsulated rosemary essential oil (4.5%) powder, T_2 - Product with spray dried encapsulated oregano essential oil (4.5%) powder, and T_3. -Product with spray dried Blended encapsulated powder. were evaluated. The samples were drawn at a regular interval of 7 days on 0, 7, 14, 21, 28 and 35 days. The storage quality was evaluated on the basis of physico-chemical (pH, Thiobarbituric Acid Reactive Substances (TBARS), Free Fatty Acids content and Peroxide value), microbiological and sensory parameters. PH of control and treatments stored at refrigeration temperature varied significantly ($P<0.05$) with the passage of storage interval. It showed an increasing trend throughout the storage period, irrespective of the type of product. The increase in pH in control product was highest than treated products. Amongst the stored products, T_3 had a lower pH value on last day of storage than other products due to protective effect of blended essential oil. Oxidative rancidity as measured by TBARS values followed a significant ($P<0.05$) increasing trend from day 1 to 35 for all treatments as well as control products, however, increase in TBARS value of treatment products was lower than control which might be due to antioxidant effect of incorporated essential oils. An increase ($P<0.05$) in FFA content during storage on the similar lines of TBARS had been observed, irrespective of treatments. The control samples had significantly higher ($P<0.05$) FFA content than all treated products during entire period of storage. Peroxide value of both the controls and treatment products followed the similar significantly increasing trend as that of TBARS values and FFA values, and the value was significantly lower ($P<0.05$) in treated products throughout the storage period. There was no significant difference in SPC amongst the control and treatments on the start of storage period, however the mean values of the standard plate count (SPC) increased significantly ($P< 0.05$) at each subsequent storage interval irrespective of treatment given. It was significantly

lower ($P<0.05$) in treated products than control and lowest value was in T_3. The mean scores for all the sensory attributes for control as well as treatment showed a decreasing trend with increase in storage period. The general appearance scores for all the treatment products and control showed a progressive significant ($P< 0.05$) decline with increase in storage period but the rate of decrease in the scores for the treatment products was lower. On 35^{th} day of storage, control product was not evaluated for all the sensory parameters since the values of microbial counts were quite high for the same. T_3 showed the highest appearance score on 35^{th} day of storage. The flavour scores also showed the similar decreasing trend as seen in the appearance scores for the control and treatment products. A decreasing trend for juiciness and texture scores was observed throughout the storage, irrespective of treatment given and the rate of decrease was lower ($P< 0.05$) in treated product as compared to control product. For overall acceptability scores, decreasing trend in the both groups i.e. control and treatment was observed with the progression of storage period. At the end of the storage period T_3 was reported to have highest overall acceptability score i.e. 6.67.

CONCLUSIONS

1. On the basis of in-vitro antimicrobial and anti-oxidant efficacy concentration of rosemary essential oil (2.5%w/v), oregano essential oil (2.0% w/v) and Tween-80 (1%) was selected for blending followed by encapsulation through spray drying.

2. Based on Encapsulation efficiency, bulk density, tapped density, solubility, wettability; combination of MD+GA(2:1) as wall material, at 18% concentration and $170^{\circ}C$ inlet temperature was selected as spray drying conditions and parameters for encapsulation.

3. On the basis of physicochemical, sensory and color attributes, 1% level of encapsulated blended essential oil powder was selected for incorporation in goat meat emulsion and formulation of chevon patties.

4. At the end of 35 days storage under aerobic packaging condition ,the chevon patties with encapsulated blended essential oils was found to be microbiologically and organoleptically acceptable than the respective control as well as one incorporated with individual essential oils.

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