

A STUDY ON EFFECT OF ENCAPSULATED RESVERATROL POWDER FOR ENHANCING THE FUNCTIONALITY OF YOGHURT

Thesis

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

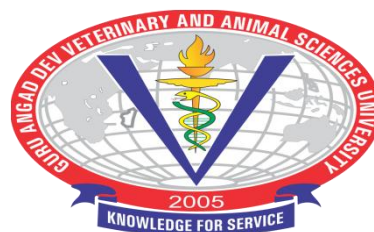
**MASTER OF TECHNOLOGY
in**

DAIRY TECHNOLOGY

(Minor Subject: Dairy Engineering)

By

**Harshita Sonarshi
(L-2016-D-01-M)**



**Department of Dairy Technology
College of Dairy Science and Technology
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Ludhiana – 141 004
2018**

CERTIFICATE – I

This is to certify that the thesis entitled, “ **A STUDY ON EFFECT OF ENCAPSULATED RESVERATROL POWDER FOR ENHANCING THE FUNCTIONALITY OF YOGHURT**” submitted for the degree of **M. Tech.**, in the subject of **Dairy Technology** (Minor Subject: **Dairy Engineering**) of the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, is a bonafide research work carried out by **Harshita Sonarhi (L-2016-D-01-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.

(Dr. S. Siva Kumar)
Major Advisor
Assistant Scientist
College of Dairy Science and Technology
Guru Angad Dev Veterinary and Animal
Sciences University
Ludhiana – 141004 (Punjab)

CERTIFICATE – II

This is to certify that the thesis entitled, “**A STUDY ON EFFECT OF ENCAPSULATED RESVERATROL POWDER FOR ENHANCING THE FUNCTIONALITY OF YOGHURT**” submitted by **Harshita Sonarhi (L-2016-D-01-M)**, to the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, in partial fulfillment of the requirements for the degree of **M.Tech.**, in the subject of **Dairy Technology** (Minor Subject: **Dairy Engineering**) has been approved by the Student’s Advisory Committee after an oral examination on the same, in collaboration with an external examiner.

(Dr. S. Siva Kumar)
Major Advisor

(Dr. Deep Narayan Yadav)
External Examiner
Food Grains and Oilseed
Processing Division,
ICAR – Central Institute of Post-
harvest Engineering & Technology
Ludhiana – 141004

(Dr. Anil Kumar Puniya)
Dean, College of Dairy Science
and Technology

(Dr. Sanjeev Kumar Uppal)
Dean, Postgraduate Studies
Guru Angad Dev Veterinary and
Animal Sciences University,
Ludhiana, India

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Harshita Sonarathi

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Major Subject : Dairy Technology

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Name and Designation of Major Advisor : Dr. S. Siva Kumar
Assistant Scientist

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ABSTRACT

Yoghurt is a well known fermented product popular in Indian sub continent and all over world. The objective of the study was to develop and characterize the functional yoghurt through fortification of encapsulated resveratrol powder. Different formulations of protein based encapsulated resveratrol powder were fortified in yoghurt range from 1-3% levels and the level of addition was optimized on the basis of sensory scores. Yoghurt fortified with 1% level (WPI/SC based wall material used for encapsulation of resveratrol powder) was comparable to control, which was further studied for sensory parameters, proximate composition, texture, colour parameters, antioxidant activity, resveratrol content and microbiological parameters during 15 d of storage. Economic viability is the most important factor to take place in the market for any food product along with its quality and sensory parameters. An attempt was made to estimate the cost of production of fortified yoghurt considering certain set of assumptions. The Break Even output was calculated as 31.28 kg of product. Pilot consumer study on the acceptability of yoghurt sample was carried out to find out the potential marketability of the product. Result findings on the basis of physico chemical characterization concluded that 1% SC based wall material used for encapsulation of resveratrol powder could be successfully incorporated in yoghurt which could serve as a potential delivery system of resveratrol for enhancing functionality of the yoghurt.

Keywords: Yoghurt, Encapsulation, Protein based wall materials, Resveratrol, Fortification, Functionality

Signature of Major Advisor

Signature of the Student

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ABBREVIATIONS

d	:	Days
g	:	Gram
gs	:	Gram second
kg	:	Kilogram
NLT	:	Not less than
NMT	:	Not more than
NS	:	Non significant
PEG	:	Polyethylene glycol
SC 1%	:	Sodium caseinate used as a wall material for encapsulation of resveratrol
SE	:	Standard Error
WPC 1%	:	Whey protein concentrate 30% used as a wall material for encapsulation of resveratrol
WPI 1%	:	Whey protein isolate used as a wall material for encapsulation of resveratrol

CHAPTER I

INTRODUCTION

India has been the leading producer of milk in the world since 1998 with a sustained growth in the availability of milk and milk products. The Indian rural economy mainly depending on the dairy activities serving as an important source of employment and income. India's dairy segment is growing due to increased demand for diversified dairy products. Increasing incomes, urbanization, dual income households and other demographic shifts are driving the demand for processed milk products. As per official website of National Dairy Development Board, India's milk production has increased from 55.6 million tonnes (1991-92) to 165.4 million tonnes (2016-17) (NDDB 2018). In India, 10% of total milk produced is converted into fermented milk products like *yoghurt*, *dahi*, *lassi* etc. This sector is growing at more than 20 percent (Singh 2011). Milk is a naturally designed gifted food source and complex mixture of specific bioactive proteins, lipids, lactose and minerals. These components play a crucial role in flavour and textural properties of milk and milk products. Amongst various dairy products, yoghurt is a popular fermented dairy product produced by the cultures consisting of *Streptococcus salivarius ssp. thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus*. This product has become famous due to the entry of a number of international and nationally organized dairy players in the market and gradually replaced the other dairy products due to its varieties and health benefits. The period between 2011-2015, the yoghurt market in India grew at a compound annual growth rate (CAGR) of 28.9% and this market is projected to touch US\$ 1 billion by the end of the year 2021. (Anon 2018a)

Health benefits associated with bioactive components has created the awareness in consumers and entrepreneurs. Amongst various polyphenols, resveratrol (trans-resveratrol; trans-3,5,4-trihydroxystilbene) is from the stilbenes family, found at relatively high levels in grape skins and also found in blueberries, raspberries, mulberries, peanuts and variety of other plant sources. Resveratrol is having lot of interest to the food and pharmaceutical fields due to its potential beneficial effects on human health including cardiovascular and neuro-protective, antioxidant, protection from infection and ischemia, antiviral, prevents against ageing, anti-inflammatory, anti-carcinogenic, and anti-obesity effects (Alves 2012). However, the major

drawback for incorporating resveratrol into functional foods is related to degradation when exposed to oxygen or light, low water solubility, low bioavailability (Hung *et al* 2006).

In food industry, there is need for the edible delivery systems to encapsulate, protect and release resveratrol when developing functional foods from it. Encapsulation may be defined as process of entrapment of one substance (active agent) within the another substance (wall materials) thereby producing particles with diameter of few nm to mm. The encapsulated substance or active agent can be called as core and the substance that is encapsulating is called as wall material, coating, shell, carrier materials etc. The characteristics of an effective wall material include good emulsion-stabilization properties, low viscosity at high concentrations, and effective redispersion behaviour in order to release the core material on rehydration. Encapsulating agents used in the food industry include carbohydrates, gums, lipids, and proteins. Carrier food systems such as lipid, protein or natural biodegradable polymer-based capsules are most often utilized for encapsulation (Chen *et al* 2006). Encapsulation is one of the most promising technologies having the feasibility to entrap bioactive compounds like resveratrol. Encapsulation enhances the bioavailability, improve controlled release and enable precision targeting of the bioactive compounds in a greater extent (Mozafari *et al* 2006). Nanotechnology has been revolutionizing the entire food system from production to processing, storage, and development of innovative materials, products, and applications (Ezhilarasi *et al* 2013 and Lopez *et al* 2006).

The health benefits of resveratrol provide a valid rationale to add the bioactive form of resveratrol into food products in order to provide the health benefits to the consumers. Also, protein may be a desirable encapsulation material for resveratrol due to the binding of resveratrol and protein which can further enhance stability. Whey protein isolate (WPI), whey protein concentrate (WPC), sodium caseinate (SC) along with maltodextrin are used as wall material to protect the core material (resveratrol) because of its emulsifying ability, water absorbing capacity, water solubility, gel forming ability, foaming ability, low cost, neutral aroma, taste, low viscosity and also protection against oxidation (Liang *et al* 2007).

Spray-drying is the commonly used drying method as the processing cost and equipment cost is lower than freeze-drying method (Dzondo Gadet *et al* 2005). The spray drying principle is employed by fine spraying a liquid into a hot gas stream, evaporating and drying the droplets in-flight and separating and collecting the solid particles from the gas stream. The design of the drying chamber may depend on the desirable droplet size and particle drying time and their residence time inside the chamber (Winsniewski 2015). Spray-drying is used for the production of encapsulated materials in the food industry yielding powders containing the encapsulated material as tiny droplets enveloped in a polymer wall matrix (Gharsallaoui *et al* 2007). Spray drying improved solubility as well as drying of nanoparticles, microencapsulation, and coating beyond its well known background on drying of liquids (Adibkia *et al* 2012).

Although a lot of literature is available on yoghurt, but little or nil scientific literature pertaining to yoghurt incorporated with milk protein based encapsulated resveratrol powder. Therefore, there is a need to optimize the manufacturing process of the product scientifically, which could be applied for its commercial level of production. Hence, this study was undertaken to observe the quality characteristics/ functionality of the developed yoghurt under refrigerated conditions. Therefore, current proposal was planned with the following objectives:

- ❖ To select and optimize the level of incorporation of protein based encapsulated resveratrol powder in yoghurt preparation.
- ❖ To study the quality characteristics of the optimized yoghurt under storage conditions.

CHAPTER II

REVIEW OF LITERATURE

This chapter includes the work done in India and abroad relevant to the present study entitled “A study on effect of encapsulated resveratrol powder for enhancing the functionality of yoghurt” has been presented under following heads:

- 2.1 Yoghurt
 - 2.1.1 Background of the yoghurt
 - 2.1.2 Definition and standards of yoghurt
 - 2.1.3 Types of yoghurt
 - 2.1.4 Value added yoghurt
 - 2.1.5 Method of manufacture
- 2.2 Major ingredients for preparation of functional yoghurt with fortification of encapsulated resveratrol powder and its health benefits.
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 - 2.2.2 Resveratrol
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 - 2.2.9 Olive oil
- 2.3 Properties of yoghurt
- 2.4 Nanoencapsulation
- 2.5 Ultrasonication
- 2.6 Spray drying as a process for nanoencapsulation

2.1 Yoghurt

2.1.1 Background of the yoghurt

Yoghurt, semi-solid fermented product has *Streptococcus thermophilus* and *Lactobacillus delbruekii ssp. bulgaricus* cultures that nurture therapeutic and promoting properties, along with proven nutritional benefits. Wikipedia, the free encyclopedia states that yoghurt, yogurt or yoghourt is a fermented milk product produced by

bacterial fermentation of milk with highly acidic in taste and has a harsh acidic flavour. It is often consumed with variety of added flavours, including fruits. Yoghurt is a good source of calcium, vitamin B₁₂, phosphorus and riboflavin. Yoghurt is a source of natural proteins and also safe for those who are having problem of lactose intolerance. It prevents osteoporosis and arthritis due to its high calcium content. It reduces the risk of high blood pressure, bad cholesterol (LDL/VLDL) and risk of heart attacks. It also helps in certain gastrointestinal problems such as constipation and diarrhoea by restoring the beneficial microbial population in the colon. Daily consumption of yoghurt can kill *Helicobacter pylori*, the bacterium responsible for most ulcers. Health benefits of yoghurt are mainly attributed to the presence of lactic acid bacteria. It provides immunity, protects cold, cough and strengthens the defence mechanism of body and strengthens the collagen in the skin and is good for our skin. For senior citizens, having more sensitive colons or intestines have lack of lactase, yoghurt is a valuable food because elderly intestines showing declining levels of Bifidus bacteria may allow the growth of toxin-producing and perhaps, cancer-causing bacteria. Meydani and Ha-Woel-Kyu (2000) reported that the preventive effects of yoghurt have been investigated on diseases such as cancer, infection, gastrointestinal disorders and asthma. It enhances the immune response, which in turn increases resistance to immune-related diseases.

2.1.2 Definition and standards of yoghurt:

According to FAO/WHO (1977) "Yoghurt is a coagulated milk product obtained by lactic acid fermentation through the action of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* from milk and milk products (pasteurized or concentrated) with or without the optional additions (skim milk powder, whole milk powder, whey powder etc) and the final product must contain viable organism in abundance.

The general definition of yoghurt was given by Tamime and Deeth (1980). The authors defined yoghurt as a product resulting from milk by fermentation with a mixed starter culture consisting only of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*.

FSSAI Standards

Yoghurt is a coagulated product obtained from pasteurized or boiled milk or concentrated milk, pasteurized skimmed milk and /or pasteurized cream or a mixture

of two or more of these products, by lactic acid fermentation through the action of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. It may also contain cultures of *Bifidobacterium bifidus* and *Lactobacillus acidophilus* and other cultures of suitable lactic acid producing harmless bacteria and if added a declaration to this effect shall be made on the label. The microorganisms in the final product must be viable and abundant. It may contain milk powder, skimmed milk powder, and unfermented buttermilk, concentrated whey, whey powder, whey protein, whey protein concentrate, water soluble milk proteins, edible casein and caseinates manufactured from pasteurized products. It may also contain sugar, corn syrup or glucose syrup in sweetened, flavoured and fruit yoghurt or fruits in fruit yoghurt. Titratable acidity as lactic acid shall not be less than 0.85 percent and not more than 1.2 percent. The specific lactic acid producing bacterial count per gram shall not be less than 10,00,000 cfu/ gm. Provided further that, the type of yoghurt shall be clearly indicated on the label otherwise standards of plain yoghurt shall apply. The yoghurt subjected to heat treatment after fermentation at temperature not less than 65 °C shall be labelled as thermised or heat treated yoghurt and shall conform to the above parameters except the minimum requirement of specific lactic acid producing count per gram. It shall conform to the following requirements:—

Table 2.1 FSSAI STANDARDS OF YOGHURT

Sr. No.	Parameters	Yoghurt	Partly skimmed yoghurt	Skimmed yoghurt
1.	Milk fat % (m/m)	Not less than 3	More than 0.5 and less than 3	0.5 (maximum)
2.	Milk solids not fat minimum, % (m/m)	8.5	8.5	8.5
3.	Milk protein % minimum, (m/m)	2.9	2.9	2.9
4.	Titratable acidity, minimum, % (as lactic acid)	0.85	0.85	0.85

Table 2.2: FSSAI Microbiological Standards of yoghurt

S. No.	Requirements	Yoghurt
1.	Total plate count	Not more than 1,00,000/gm
2.	Coliform count	Not more than 10/gm
3.	<i>E. coli</i>	Absent in 1gm
4.	<i>Salmonella</i>	Absent in 25 gm
5.	<i>Shigella</i>	Absent in 25 gm
6.	<i>Staphylococcus aureus</i>	Not more than 100/gm
7.	Yeast and mold count	Not more than 100/gm
8.	Anaerobic spore count	Absent in 1 gm
9.	<i>Listeria monocytogenes</i>	Absent in 1 gm

2.1.3 Types of yoghurt:

Types of yoghurt on the basis of total solids content:

Full fat yoghurt has fat percent more than 3. The survival of *Bifidobacterium bifidum* BBI and *Lactobacillus acidophilus* LAI in reduced-fat (liquid) and full-fat (set) yoghurt produced with two commercial lactic starter cultures (SID and SISD) was investigated. The results showed that the full fat yoghurt was a more inhibitory medium than the reduced fat one, especially for *B. bifidum* BBI (Vinderola *et al* 2000). Medium fat yoghurt has fat percent in between 0.5 to 3. Low fat yoghurt have fat percent less than 0.5. The perceived creaminess resulted from in-mouth melting of these amyl maltase-treated starch (ATS) domains due to a combined effect of their physical melting and hydrolysis by amylase present in the saliva (Alting *et al* 2009). Balkan yoghurt has fat % in between 4.5 to 10.

The milk solids content for yoghurt ranges from around 9% for skim milk yoghurt to 20% for certain types of concentrated yoghurt. Many commercial yoghurt products have milk solids contents of 14-15% (Tamime and Robinson, 1999).

The minimum milk solids not-fat content required in standards or regulations in many countries ranges from 8.2 to 8.6% (Tamime and Robinson 1999). Codex regulations for yoghurt indicate that the minimum milk protein content is 2.7%

(except for concentrated yoghurt where the minimum protein content is 5.6% after concentration) and the maximum fat content is 15% for concentrated yoghurt (Codex 2008).

Type on the basis of texture/consistency: Set Yoghurt and Stirred Yoghurt

Set yoghurt: This type of yoghurt has a thick texture prepared by using warm cultured milk mixture is poured into containers then incubated without any further stirring. Three stabilizers gelatin, pectin and sodium alginate were used at different rate 0.2%, 0.4% and 0.6% w/w respectively for preparation of yoghurt (Kumar *et al* 2004). The addition of whey protein isolate (WPI) on the consistency and microstructure of set yoghurt was studied, the addition of WPI gave weaker texture of the set yoghurt and less coherent microstructure (Guggisberg *et al* 2007).

Stirred yoghurt: This type of yoghurt is slightly thinner than the set yoghurt, prepared by warm cultured milk mixture is incubated in a large vat, cooled and then stirred for a creamy texture, often with fruits or other flavourings added in cold beverages or in desserts. The stirred yoghurt fortification with iron distressed bees honey and gelatin 5 and 10 mg/100g yoghurt gave positive effect on overall acceptability scores in comparison with 15 and 20 mg iron/ 100g yoghurt (Elhamid *et al* 2016).

2.1.4 Value added yoghurt:

Dietary fiber: Yoghurt was prepared with incorporation of Wine grape pomace as a source of antioxidant dietary fibre and stored at 4 °C for 3 weeks. From the results, it was found that product had increased dietary fibre and polyphenol contents. This delayed the lipid oxidation during storage and increased the acceptability of the product. (Tseng *et al* 2013).

Frozen dessert: It is a type of frozen dessert made with yoghurt, usually regarded as a low-fat alternative to ice cream.

Herbs: The addition of menthe piperita, anethum graveolence and ocimum basilicum in yoghurt was done and effect on the inhibition of enzyme relevant to hypertension and type II diabetes was analysed. The herbal yoghurt has high antioxidant activity with beneficial effects on enzymes important in the management of hypertension and diabetes mellitus as compared to plain yoghurt (Amirdivani 2007). Herbal yoghurt was prepared with pre-treated herbs, namely tulsi leaf, pudina leaf and coriander leaf.

Tulsi based herbal yoghurt exhibit maximum beta galactosidase activity compared with control yoghurt (Chowdhury *et al* 2009).

High protein yoghurt: The high protein strawberry yoghurt was prepared with whey protein isolate and the low-protein yoghurt consisted of a close-to-commercial strawberry yoghurt and the result shows that higher protein versions of the test meals decreased the hunger more than the lower protein version (Vadewater *et al* 1996). Non-fat yoghurt was manufactured from skim milk fortified with a new high milk protein powder up to 5.6% protein. The added protein assisted in providing a firm body and minimal whey separation without the use of stabilizer (Mistry *et al* 1992).

2.1.5 Method of Manufacturing:

Milk was heated to 85°C/15 min. The heated milk was cooled to 42°C. Then culture was added and mixing was done. The inoculated milk was incubated at 42°C for 4-5 hrs, once the required acidity 0.85% LA and pH 4.3-4.2 was reached the yoghurt was transferred to 5°C (Tamime and Robinson 1999).

Stabilizers such as pectin and gelatin are often added to the milk base to maintain the appropriate yoghurt properties including texture, mouth feel and appearance (Tamime and Robinson 1999). The use of stabilizers may help in prevention of whey separation and lessen the batch to batch variation. Milk is typically homogenized using pressures of 2000 psi in first and 500 psi in second stage at a temperature range between 55 and 65°C. The use of homogenization prevents fat separation (creaming) during fermentation or storage, reduces whey separation, increases whiteness, and enhances consistency of yoghurts (Vedamuthu 1991). Homogenized milk fat globules act like protein particles due to the presence of protein on the fat surface (Walstra 1998).

The heat treatment of milk is also used to destroy unwanted microorganisms, which provides less competition for the starter culture. Yoghurt starter cultures are sensitive to oxygen so heat treatment helps to remove dissolved oxygen to assist the starter growth. Considering the economy of the process, pasteurization of milk 85°C for 15min is sufficient to kill the microorganisms and also there is non significant improvement observed with increase in intensity of heat treatment (Tamime and Robinson 1999).

After heat treatment, the milk base is cooled to the incubation temperature used for growth of the starter culture. An optimum growth temperature of the

thermophilic lactic acid bacteria, i.e. *Streptococcus salivarius subsp. thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* is around 40-45°C. Bacterial fermentation converts a part of lactose into lactic acid, which reduces the pH of milk. During acidification of milk, the pH decreases from 6.7 to ≤ 4.6 . Gelation starts occurs at pH 5.2 to 5.4.

When yoghurt has reached the desired pH (e.g., 4.6) for production of set yoghurt it was often blast chilled to $<10^{\circ}\text{C}$ then transferred to the refrigerated cold store to reduce further acid development (Tamime and Robinson 1999). For stirred yoghurts, cooling is first performed by agitation in the jacketed fermentation vat and the product is sheared and smoothed by devices like back-pressure valves, high shear devices or sieves. Satisfactory yoghurt texture and flavour were achieved by cooling to 4-5°C.

2.2 Major ingredients for preparation of yoghurt with fortification of encapsulated resveratrol powder and its health benefits.

2.2.1 Milk and culture

The factors influencing the yoghurt manufacture includes standardization of milk, homogenization, heat treatment, inoculation level of the starter culture, incubation/fermentation, cooling and storage. The body and texture of yoghurt depends largely on the composition of milk employed in its manufacture. The Fat and SNF contributes to enhancement in taste of the yoghurt with improved consistency and viscosity with reduced syneresis. It improves the flavour, rheological properties and overall acceptability of yoghurt (Guirguis *et al* 1984). Cow's milk is preferred for preparing yoghurt as it has low fat. Milk powders, including non-fat dry milk, whey protein concentrates, or milk protein concentrate, can be blended with the milk using a powder dispersion unit. The stage of packaging depends upon the type of yoghurt that is manufactured i.e., set, stirred, drinking or frozen.

2.2.2 Resveratrol

Resveratrol (3,4',5- trihydroxy stilbene) is a nutraceutical due to its exciting pharmacological potential for curing many diseases. It is a phytoalexin found in many plants including grapes, peanuts, berries and the root of *polygonum cuspidatum*. Resveratrol polyphenol, acts as an antioxidant and a free radical scavenger. It exists as trans and cis isomers, both of which are present in biological materials in wide concentration range. Resveratrol has antibacterial and antifungal

properties and induces platelet hypoaggregation in rats. Resveratrol protects the liver from lipidic peroxidation and inhibits the oxidation of low density lipoproteins (LDL), modulating their metabolism. The cis-isomer have potential anticancer activity, as do the trans isomers, by inhibiting protein-tyrosine kinase (Jayatilake *et al* 1993). The use of resveratrol in food industry is currently limited due to its poor water solubility, low bioavailability and chemical instability.

Molecular formula: C₁₄H₁₂O₃

Molecular weight: 228.24

Appearance: Fine crystal powder

Colour: white

Resveratrol is highly soluble in ethanol 50mg/ml and dimethyl sulphur oxide (16mg/ml) moderately soluble in triacylglycerol oils (0.18 mg/ml). Resveratrol has wide solubility ranging from 0.05 mg/ml in water to 374 mg/mL in polyethylene glycol 400 (PEG-400) (Hung *et al* 2009). In wine concentration of resveratrol varies as follows: red wine contain between 0.2 to 5.8 mg/l, depending upon the grape variety, whereas white wine contain approx 0.68mg/l. Red wine contain more trans resveratrol than white wine, where as white wine has higher concentration of cis resveratrol (Feijoo *et al* 2008).

Health benefits

Anticancer effect: Resveratrol selectively suppresses the transcriptional activation of cytochrome p450 1A1 and inhibit the formation of carcinogens induced preneoplastic lesions in the mouse mammary organ culture model, promoted mouse skin tumor in a two stage model.

Treatment of lung cancer: Resveratrol is reported to have protective effect against lung cancer. It shows the arrest of A549 cells in the G1 phase of the cell cycle. Resveratrol is a potent inhibitor of A549 lung cancer.

Suppresses breast cancer:

Resveratrol is a phytoestrogen that displays estrogen-like agonistic and antagonist activity which appears to mediate some of its action by modulating the estrogen machinery and acts as an anti-breast cancer agent (Basly *et al* 2000).

Prevention of cardiovascular diseases:

Resveratrol reduce the myocardial damage during ischemia-reperfusion, modulation of vascular cell function, inhibition of LDL oxidation and suppresses the

platelet aggregation. It inhibits lipids peroxidation of low density lipoprotein (LDL), prevents the cytotoxicity of oxidized LDL and protects cell against lipid peroxidation. Resveratrol contains highly hydrophilic and lipophilic properties provide a more effective protection than other antioxidants, such as vitamin C and vitamin E. The resveratrol has direct inhibitory action on cardiac fibroblast and may inhibit the progression of cardiac fibrosis. Resveratrol initially used for cancer therapy and also shown beneficial effects against most degenerative and cardiovascular diseases. It also shows beneficial effects against atherosclerosis, hypertension, ischemia/reperfusion, heart failure due to diabetes, obesity, and aging.

Anti-platelet aggregation: The clumping of blood platelets cause blood clotting and thrombosis. Resveratrol is active in preventing platelets from bunching up together and keeps the blood smoothly flowing through arteries. Recent studies showed that resveratrol freely dilates blood vessel bringing down the blood pressure.

Anti ageing action: Caloric restriction extends life by reducing cell damage from free radicals, which are generated when cells burn nutrients in the mitochondria to produce energy. Caloric restriction seems to trigger very specific cellular mechanism of self preservation whose biological role is to extend survival of the organism until food intake increases sufficiently to ensure successful reproduction.

Brain function: Resveratrol has been shown to facilitate the breakdown of beta-amyloid, which is associated with Alzheimer's disease, via specific mechanisms unrelated to antioxidant effects and to protect against beta-amyloid toxicity *in vitro* (Savaskan *et al* 2003). Resveratrol has also been shown to protect the brain against the effects of traumatic injury in a rat model (Ates *et al* 2007).

Bioavailability of resveratrol

Bioavailability is a degree to which or rate at which a drug or other substances is absorbed or becomes available at the site of physiological activity after administration. In humans, 24.6% of the oral dose administered appeared in the urine, including metabolites, whereas after intragastric administration to rodents, only 1.5% of resveratrol reached the plasma compartment (Mohammad *et al* 2007).

Recommended Doses:

As per Australian Journals 30mg of resveratrol consumed daily without any side effects, whereas, University of Pittsburgh Medical Center reported that a reasonable dosage of resveratrol is approximately 500mg per day. However, there is

currently no established dosage recommendation for resveratrol. The results of several studies conducted by the New York Langone Medical Center suggested daily doses of 500mg of resveratrol might be safe and potent.

2.2.3 Whey protein isolate

Whey proteins are the by products of cheese manufacturing having superior gelling and emulsifying properties. The health benefits associated with whey protein include antimicrobial activity, inhibition of angiotensin-converting enzyme and anti carcinogenic activity among others. The composition of WPI powder include 92.0% protein, 4.5% moisture, 2.0% ash, 1.0% fat and 0.5% lactose. But, manufacturing technology creates varying composition and functional properties in the end product. WPI has good emulsifying, fat-binding, and water-binding or thickening properties added to products to take advantage of its gelling and whipping properties.

WPI is soluble or forms a stable colloidal dispersion at ambient temperature and under all pH conditions. WPI will provide low viscosity when used in liquid products. WPI is very stable across the entire food pH range depends upon the conditions.

The spray dried powder was prepared from blueberry pomace extract and whey proteins. The resulting microcapsules having 48.5 μm in diameter, 5% moisture content and contained 1.32 mg cyanide-3-O-glucoside(C3G), 2.83 mg gallic acid equivalents(GAE) and 48.52 nmol Fe(II) equivalents per gram powder. Storage tests revealed first order degradation kinetics for monomeric anthocyanins, a two fold increase in total phenolics and slight increase in antioxidant capacity. Exposure to light was comparable to storage at 37°C, but slightly more severe in decomposing anthocyanins. The spray dried encapsulated powder could be used as a suitable health promoting food ingredient (Flores *et al* 2014).

2.2.4 Whey protein concentrate

Whey proteins are often used in food emulsion systems because of their ability to stabilize oil-in-water emulsions. Whey Protein Concentrates (WPC) are powders made by drying the retentates from ultrafiltration (UF) of whey. They are described in terms of their protein concentration, protein content as percentage of dry matter, which ranges from 25 to 80%, used extensively as functional and nutritional ingredients in medical, pharmaceutical, and food products.

Whey proteins have two types of properties viz. nutritional and functional. As far as nutritional attributes are concerned, the whey protein concentrates are rich in essential amino acids such as lysine, tryptophan, cystine, and methionine enabling its use in infant food and aged people diet as well as in enrichment of cereals. Whey proteins are known for their high solubility over a wide range of pH, (lower the pH, higher the solubility), better aeration properties, ability to form gels at high temperature, and better water and fat binding properties.

The important functional properties of the whey protein concentrate as follows:

The solubility of whey protein affects the other functional properties like gelling, foaming, and emulsifying properties. When the temperature increases, the solubility of whey protein is lost since they are heat sensitive. Among them, the immunoglobulins are more sensitive as they are denatured at a temperature of 70°C. The proteose-peptone component is the most heat resistant among the entire whey protein component. Denaturation by heat is nothing but breakage of sulphur bridge within the molecule resulting in unfolding and insolubility.

The maximum water absorption by the whey protein concentrate occurs when the exposure is between 5-10 min. But sodium caseinate and soy protein isolates are capable of absorbing water better than the whey protein concentrates.

Heating causes the unfolding and higher the temperature, the gel formed is stronger. If 80% concentrated WPC is heated, gel formation can be noticed on cooling.

Denatured whey proteins have poor whipping properties and to have a better whipping property, severe heat treatment of whey proteins should be avoided. But mild heat treatment tends to favour or improve the whipping ability of the whey proteins.

The surface properties of the whey proteins make them good emulsifying agents. The emulsifying ability or capacity directly depends on the solubility of the whey protein. Hence the factors that affect the solubility in turn regulate the emulsion formation.

2.2.5 Sodium caseinate

Milk proteins especially caseinates are good emulsifiers and hence used as an ingredient in a wide range of formulated food emulsions. Studies have been made of changes in particle sizes, surface coverage and composition, stability of emulsions formed with soya oil and sodium caseinate. (Srinivasan *et al* 2000)

2.2.6 Maltodextrin:

Maltodextrins, low converted products of starch hydrolysis, are used in various emulsions to provide desirable viscosity, texture and mouth feel. In order to assess behaviour of such emulsions in process of production (emulsification) as well as end products in application it is necessary to investigate emulsification dynamics, globule size changes and rheological behaviour. From the results, it was found that certain specific maltodextrin characteristics such as dextrose equivalent-measure of the degree of starch polymer hydrolysis (DE Value) i.e. size of maltodextrin molecules significantly influence dispersion and viscous properties and stability of emulsions. (Baucal *et al* 2003). Maltodextrins are frequently used as bulking agents; however, when used at sufficiently high concentrations, maltodextrins have the ability to bind water, thereby contributing significantly to mouthfeel and viscosity (Lucca and Tepper 1994). Maltodextrins can also act as a carrier agent, which protects encapsulated ingredients from oxidation

2.2.7 Soy Lecithin

Soy lecithin offers a multifunctional, flexible and versatile tool. It is probably best known for its emulsifying properties and also non toxic. The unique lipid molecular structure makes lecithin useful for pharmaceutical applications. The unique surface-active properties of lecithin make it ideal for emulsification. An emulsion is a stable blend of materials that do not mix easily, tending to separate. Lecithin can decrease mixing times and improve results when combining dissimilar ingredients. Whether water-in-oil or oil-in-water emulsion, the lecithin should be dispersed in the oil phase first. For o/w emulsions, lecithin functionality can be achieved anywhere from 5%-10% of the weight of oil. In w/o emulsions, lecithin incorporation is substantially lower at 1%-5% of weight of oil. The influence of emulsifiers and stirring on catastrophic inversion (O/W to W/O) was studied. Emulsions were prepared with different stirring rates, using soy lecithin and Tween 80, at 2 and 5 wt%. The aqueous phase was distilled water with 1 wt% NaCl and the oil phase was soy oil. Rheological analysis showed that, with a higher concentration of emulsifier, it is better to use Tween 80 when lower viscosity is desired, while soy lecithin is more appropriate for higher viscosity products. The oscillatory tests showed that while the emulsions prepared using Tween 80 exhibited concentrated solution behaviour, those prepared with soy lecithin exhibited strong gel behaviour (Zuge *et al* 2003).

2.2.8 Poly ethylene glycol:

Polyethylene glycol (PEG) is formed by the reaction of ethylene oxide and water under pressure in the presence of a catalyst. Liquid PEGs are soluble in acetone, alcohols, benzene, glycerin, and glycols. In tablet formulations, PEGs of a high molecular weight can enhance the effectiveness.

2.2.9 Olive oil

Olive oil is rich in monounsaturates and has a resistance to oxidative changes because of its moderate and the presence of nutrients and non nutrients antioxidants. The effect of gallic acid, catechin and quercetin on physical and chemical stability of olive oil-in-water emulsion was investigated. From the results it was confirmed that the polarity is not only the parameter to determine efficiency of antioxidants in emulsions but other variables, such as its antioxidant activity in terms of capacity and rapidity in donating an hydrogen atom, can effects its protective role towards lipid auto-oxidation in emulsions (Di Mattia *et al* 2009). Olive oil contains natural anti-oxidizing agents such as phenols and vitamin E. When heated, olive oil is the most stable fat, which means it stands up well to high temperatures.

2.3 Properties of yoghurt

Physical and sensory properties of yoghurt

The physical and sensory properties of yoghurt gels are highly influenced by the protein content and the total solids content of the yoghurt milk. The addition of protein improves water holding capacity, decreases the synerisis effect and stabilizes the pH of the samples during storage. The milk supplementation (whey, casein hydrolysate and milk protein) on the acidification and microbiological stability of fermented milks showed that acidifying activity was greatly improved with casein hydrolysate with a reduction of the fermentation time by about 55% by comparison with the other supplementation (Oliveria *et al* 2001). Increasing milk total solids from 16 to 23% had significant effect on decreasing rate of pH during fermentation (Ozer *et al* 1998). The increase in milk fat content influences the growth and activity of starter. (Mahdian *et al* 2006). Balasubramanyam and Kulkarni (1991) reported that increased sugar level in the yoghurt reduces the viscosity, curd tension and wheying off. The sugar impedes the swelling of protein micelles thus affecting the gel strength of the yoghurt. The increased solids content in yoghurt milk as a result of fortification also creates increased buffering that requires additional acid development by the starter

cultures to achieve a similar pH target. The use of sucrose increases the total solids of the mix and strengthens the gel network.

Rheological properties of yoghurt

Yoghurt exhibits time-dependent shear thinning behavior but is not a true thixotropic material, since structural breakdown due to shear is not completely reversible once the shear stops. The influence of carrageenan and total solids on the rheological and physicochemical properties of lactic beverages and reported that all lactic products showed a non-Newtonian behavior with thixotropy. (Penna *et al* 2006). The influence of four types of fiber on the flow and sensory characteristics of yoghurt and reported that the viscosity was affected by the fiber type; however, yoghurts had a very good acceptability (Dello *et al* 2004). The elevation of protein content leads to increased thixotropy of the yoghurt and incorporation of milk fat in emulsified state results the yoghurt an increased viscoelastic properties (Houze *et al* 2005). Heat treatment of milk for 15 min at $\geq 80^{\circ}\text{C}$ results in significantly increased denaturation of β -lactoglobulin compared with milk heated at 75°C for a similar time. The extent of denaturation of whey proteins during the heat treatment of milk affects the firmness and viscosity of acid milk gels. (Lucey *et al* 1997).

The main processing parameters influencing the yoghurt texture are fortification level and material(s) used, stabilizers type and their usage levels, fat content and homogenization conditions, milk heat treatment conditions, starter culture (type, rate of acid development and production of exopolysaccharides), incubation temperature (influences growth of starter cultures, gel aggregation, bond strength), pH at breaking, cooling conditions, handling of product post manufacture (e.g. physical and temperature abuse), (Lucey 2004)

Colour profile analysis of the products

Colour measurement is an important tool in the hands of food technologists for determining and monitoring quality of the food product. Colour measurement need to quantify the colour changes, which will bear significant correlations with process parameters or sensory parameters.

In several dairy products, Maillard browning and caramelisation play an important role in their colour and appearance .In heat desiccated milk products like *kunda* and *kalakand*, the brown colour is a result of continuous heating of milk or high moisture khoa mixed with calculated amount of sugar. (Kulkarni *et al* 2001).

Yoghurt color was affected by the addition of DF or WB. The date fiber had a brownish color, whereas the wheat bran had a yellowish color. Yoghurts fortified with DF or WB had significantly higher a^* and b^* values and lower L^* values compared with the control yoghurt. Increasing the DF level increased a^* and b^* values and decreased L^* values significantly. Yoghurts fortified with DF had significantly lower L^* values and higher a^* values compared with WB yoghurt. Yellowness of the yoghurt depends on the level of DF. Yoghurt fortified with 3% DF had similar b^* values as WB yoghurt. Yoghurt fortified with 1.5% DF had significantly lower b^* values compared with WB yoghurt, whereas yoghurt fortified with 4.6% had significantly higher b^* values. yoghurt colour is dependent on the color of the fiber source. (Hashim *et al* 2009).

Effect of storage on properties of yoghurt

Effect on sensory attributes of yoghurt

Farooq and Haque (1992) reported that during storage the appearance of yoghurt was affected and not acceptable which ultimately deteriorate the quality of yoghurt may be due to the growth of some yeasts and mould during storage. The decrease in flavour is correlated with the proteolytic activity of bacteria and the production of higher acidity. Loss of flavour is attributed to fat and protein degradation and development of slight sharp flavour produced by coliform bacteria, clostridiums spp. and other organisms. The loss of taste in yoghurt samples may be due to development of acidity, oxidation of fat or proteolysis of proteins. Spoilage of cultured dairy products is primarily caused by excessive fermentation, enzymatic breakdown or contamination with undesirable microorganisms. The low pH of yoghurt may offer a selective environment for growth of acid tolerant yeasts and moulds. Psychrotropic organism may also pose a serious problem ,the defects like whey separation, ropiness high acidity, gas formation and curdy body formation developed during storage at low temperature.

Effect on Titratable acidity, pH values and syneresis values

The effect of wheat bran (natural and toasted) and flavourings (pineapple and piña colada) on yoghurt quality was studied by Aportela-Palacios *et al* (2005). The pH increased and syneresis decreased with increasing fiber (1.5, 3.0, and 4.5% by weight). Vahedi *et al* (2008) reported the increase in pH value after 14 days in strawberry and 21 days in apple yoghurt during storage. Microorganism's activity

caused pH decrease in fruit yoghurt. Yeasts also used sugar and organic acids and so pH decreased. As sugar sources get exhausted, begin to consume proteins, producing some products and results in pH increase.

Syneresis is the separation of the liquid phase in gels and is undesirable in fermented milks. Harwalkar and Kalab (1983) reported that the major factors contributing syneresis in yoghurt include low acidity (pH >4.6) or with high acidity (pH <4.0) high incubation or storage temperature, agitation at the time of manufacture or final product transportation, mixture having low total solids content (protein and/or fat), milk treatment at low heat, excessive heat treatment of the final mix, milk is not homogenized prior to fermentation, acidification at rapid rate, sloping wall containers or an excessive height to width ratio. Heating of milk destined for yoghurt production is one of the processing measures, which prevents the development of syneresis in the finished product.

Microbiological Aspect

Fruit-flavoured yoghurt was made by adding 2.5, 5.0, 7.5 and 10.0% mulberry pekmez (MP) into milk. The titratable acidity, pH, viscosity, whey separation and lactic acid bacteria (LAB) counts were determined at weekly intervals for 28 days. The pH range of the MP yoghurts was 4.65–5.57 and the pH of the plain yoghurt was 4.47 ($P < 0.05$). The addition of MP led to an increase in the fermentation time and a decrease in the viscosity of the yoghurts. Statistically significant differences were found between the plain and MP yoghurts in terms of pH (4.01 and 4.35), viscosity (5429 and 3175 cP) and number of LAB (7.07 and 6.48 log cfu). During storage, the titratable acidity, viscosity and LAB counts of MP yoghurts were lower and the whey separations higher than those of controls (Celik *et al* 2006). Study was to develop a predictive model to quantify the spoilage of yoghurt with fruits. Product samples were stored at various temperatures (5–20 °C). Samples were subjected to microbiological (total viable counts, lactic acid bacteria-LAB, yeasts and moulds) and physico-chemical analysis (pH, titratable acidity and sugars). LAB was the dominant microflora. Yeasts population increased at all temperatures but a delay was observed during the first days of storage (Mataragas *et al* 2010).

2.4 Nanoencapsulation

Nanoencapsulation of bioactive compounds has versatile advantages for targeted site-specific delivery and efficient absorption through cells (Ezhilarasi *et al* 2012). Nanotechnology has emerged as one of the most promising scientific fields of

research in decades. It deals with the production, processing, and application of materials with sizes less than 1,000 nm (Sanguansri and Augustin 2006). The application of nanotechnology to the food sector could generate innovation in the macro scale characteristics of food, such as texture, taste, other sensory attributes, coloring strength, processability, and stability during shelf-life, leading to a great number of new products. Nanotechnology can also improve the water solubility, thermal stability, and oral bioavailability of bioactive compounds (Huang *et al* 2010). Nanoparticles are colloidal-sized particles with diameters ranging from 10 to 1,000 nm and are expressed both as nanocapsules and nanospheres (Lopez *et al* 2006).

Wall and core materials used in nanoencapsulation

The wall system is designed to protect core material from factors that may cause its deterioration, to prevent a premature interaction between the core material and other ingredients, to limit volatile losses, and also to allow controlled and sustained release under desired condition (Shahidi *et al* 1993).

Techniques

Nanoemulsions, being non- equilibrium systems, cannot be formed spontaneously and consequently it needs energy input, generally from mechanical devices or from the chemical potential of the components. Therefore, nanoemulsion formation is generally achieved using high-energy emulsification methods like high shear stirring, high-speed or high-pressure homogenizers, ultrasonicator, and microfluidizer. These methods supply the available energy in the shortest time and possess the most homogeneous flow to produce the smallest droplet sizes . Microfluidisation was an efficient emulsification technique, yielding less surface oil on encapsulates, but when energy input increased beyond moderate pressure it leads to over-processing due to recoalescence. Comparably, ultrasonication was also a better emulsification technique and increasing energy input resulted in the reduction of emulsion droplet size with minimum recoalescence. High-pressure homogenization yielded smaller droplet sizes with the temperature increased up to 50 °C than high-speed homogenization. All these emulsification methods were capable in reducing the droplet size to a larger extent. Hence, the emulsification technique proved to be one of the effective nanoencapsulation techniques but depends on a good drying technique to produce these encapsulates in powder form. High bioavailability of active compounds which is important for the delivery of nutrients and active substances. They offer good

formulation properties since they are transparent and their submicron/nano size droplets cause a smooth and creamy mouth feel. Thereby, the production of stable nanoemulsion is an omnipresent task for the food industry.

2.5 Ultrasonication

Ultrasonic technology has been proven effective at creating encapsulating particles and droplets with specific physical and functional properties. The ultrasound is being carried out in various areas of food technology namely crystallization, freezing, bleaching, degassing, extraction, drying, filtration, emulsification, sterilization, cutting, etc.

Sonication is the process of converting an electrical signal into a physical vibration that can be directed toward a substance. The primary part of a sonication device is the ultrasonic electric generator. This device creates a signal (usually around 20 KHz) that powers a transducer. This transducer converts the electric signal by using piezoelectric crystals or crystals that respond directly to the electricity by creating a mechanical vibration. This vibration, molecular in origin is carefully preserved and amplified by the sonicator, until it is passed through to the probe. The sonication probe transmits the vibration to the solution being sonicated. This probe is a carefully constructed tip that moves in time with the vibration, transmitting it into the solution. The probe moves up and down at a very high rate of speed, although the amplitude can be controlled by the operator and is chosen based on the qualities of the solution being sonicated.

Mechanism: The rapid movement of the probe creates an effect called cavitation. Cavitation occurs when the vibrations create a series of microscopic bubbles in the solution, pockets of space wedged between the molecules that form and then collapse again under the weight of the solution, sending out tiny shockwaves into the surrounding substance. Thousands of these bubbles forming and collapsing constantly create powerful waves of vibration that cycle into the solution and break apart the cells.

There are different-sized probe tips based on what sort of sonification process is desired. A very small tip will create excellent cavitation effects and easily disrupt surrounding cells, but it will have a limited area of effect based around the probe itself. Larger tips can reach a greater quantity of the solution but will not produce such an intense reaction.

Ultrasonic waves are produced by following methods.

Magneto-striction generator or oscillator

Piezo-electric generator or oscillator

The food industry as well as the pharmaceutical industry offer manifold possibilities for the use of ultrasonics

- Activation (acceleration) of an enzyme reaction in liquid foods
- Mixing, Homogenizing and Emulsifying of oil/fat in a liquid stream
- Dispersion of a dry powder in a liquid
- Degassing
- Deactivation of enzymes
- Microbial inactivation (preservation)
- Crystallisation

2.6 Spray drying as a process for nanoencapsulation:

Spray drying is the most common and the cheapest technique to produce nanoencapsulated food materials. Equipments are readily available and production costs are lower than the most other method. Compared to freeze drying, the cost of spray drying method is 30-50 times cheaper. Encapsulation by spray drying mainly involves four steps: preparation of emulsion or dispersion; homogenization of dispersion; atomization of in feed emulsion; and dehydration of atomized particles.

Spray drying involves the process of transformation of feed (solution) into a dried particulate form by spraying the feed into a hot drying medium. This technique is reported to be considered as a suitable process for consolidating nanoparticles into macroscopic compacts and submicron spherical powders with nanometer-scale properties (Okuyama and Lenggoro 2003). Spray drying is an efficient drying technique to stabilize the nanocapsules. Compared to freeze drying, it is more economical and fast and is a single-step drying method. It yields uniformly spherical shaped particles, which offer complete protection of the core material being encapsulated, it yields particles of micron size on drying the nanoemulsions and nanosuspensions.

Working principle of spray drying

Liquid atomization can be carried out by pressure or centrifugal energy. Used atomizer includes pneumatic atomizer pressure nozzle, spinning disk configuration and two fluid or ultrasonic nozzles. The goal of this stage is to create a maximum heat

transferring surface between the dry air and the liquid in order to optimize heat and mass transfers. The choice upon the atomizer configuration depends upon the nature and viscosity of feed and the desired characteristic of the dried product. The higher the provided energy is, the finer are the formed droplets. For the same energy amount, the size of formed particles increases with increasing feed rate.

With respect to the atomizer placement compared to the hot air spread, one can distinguish concurrent drying and counter current drying. In co-current process the liquid is sprayed in the same direction as the flow of the hot air through the apparatus, hot air inlet temperature is typically 150-220°C evaporation occurs instantaneously and for which dry powders will be exposed to moderate temperature (typically 15-18°C) which limits thermal degradation. Whereas during counter current drying, the liquid is sprayed in the opposite direction of the flow of hot air and for which the dry product is exposed to high temperature which limits this process for the thermo sensitive products.

When droplets-hot air contacts, balances of temperature and vapour partial pressure established between liquid and gas phases. Thus, heat transfer is carried out from air towards the product as a result of temperature difference whereas water transfer is carried out in the opposite direction due to vapour pressure difference.

The separation is done often through a cyclone placed outside the dryer which reduces product losses in the atmosphere: most dense particles are recovered at the base of the chamber, while the finest one pass through the cyclone to be separated from humid air. In addition to cyclone, spray dryers are commonly equipped with both filters called “bag houses” that are used to remove the finest product, and chemical scrubber to remove the remaining powder and any pollutants.

CHAPTER III

MATERIALS AND METHODS

The present study entitled, “**A study on effect of encapsulated resveratrol powder for enhancing the functionality of yoghurt**” was conducted with the systematic approach to incorporate encapsulated resveratrol powder for the preparation of functional yoghurt. The experiments and analysis of samples were conducted in the Experimental Dairy plant and in the Dairy Technology laboratory, College of Dairy Science and Technology, GADVASU, Ludhiana. The details of the materials and the methods were presented in this chapter.

3.1 PROCUREMENT OF RAW MATERIALS

Selection of ingredients

Selection of good quality ingredients is very important factor for any research investigation followed by good manufacturing practices. Hence, good quality raw materials were selected for use in the present study.

Raw milk

Fresh, clean and good quality mixed (cow and buffalo) raw milk was obtained from Experimental Dairy Plant, College of Dairy Science and Technology, GADVASU Ludhiana. This milk was standardised by using skim milk to adjust the fat and SNF percent to 3% and 10% respectively. Skim milk was obtained by separating mixed milk in cream separator.

Yoghurt starter culture: NCDC 144 (*L. bulgaricus* and *S. thermophilus*) was procured from National Collection of Dairy Cultures (NCDC), ICAR-NDRI, Karnal, India

Materials required for encapsulation

Milk protein based wall materials like whey protein isolate, whey protein concentrate (30%), sodium caseinate purchased from modern dairy plant, Karnal and Mahaan Proteins Ltd, New Delhi. Resveratrol was obtained from Herbo nutra Ingredients, Bangalore. Soy lecithin from DuPont India Pvt Ltd, Gurgaon, Haryana. Olive oil and Maltodextrin (SRL) were purchased for preparation of encapsulation of resveratrol component.

Packaging materials:

The packaging material polystyrene cups were procured from CODST Dairy Plant, Ludhiana.

3.2 EQUIPMENT DETAILS:

Processing equipment and accessories: induction heater, refrigerator, incubator, laminar flow chamber, autoclave, water bath

Ultra sonicator: Sonics (USA) Vibra Cell TM was used to convert the emulsion into nanoemulsion. Model: 750 Watt ultrasonic processor VCX 750 (750 watt).

Spray dryer: Spray dryer of S M Scientific was used for conversion of nanoemulsion into powder. The stable nanoemulsion with different formulations were subjected to spray drying viz operating parameters as follows

SMST –Tall type Spray Dryer.

Capacity of the Spray Dryer: 2-3 litre water evaporation /Hr.

Inlet Temperature levels (150,160,170°C)

Atomizer Air Pressure (2/2.5/3)

Feed Pump RPM (24/30/36)

Particle Size analyser: Particle size distributions, Mean droplet diameters, and polydispersity index (PDI) were measured using dynamic light scattering instruments (Zetasizer Nano ZS, Malvern Instruments, England).

Digital pH meter: Digital pH meter (Make- Mettler Toledo) was used to measure pH during the investigation. The electrode assembly was calibrated against standard buffers of pH 7.0 and 4.0 before it was used for measuring pH of the product.

Texture Analyzer: TA.XT plus (Stable Micro Systems, UK) was used for measuring hardness, consistency, cohesiveness and index of viscosity of control and experimental yoghurt samples.

Colour Flex Colorimeter (Hunter lab, Reston, Virginia) supplied along with the universal software Easy Match QC (version 4.62) and the results were expressed in terms of L*, a*, & b*.

Automatic Moisture analyser: Medler Toledo, USA was used to analyse the moisture content of powder samples.

Kjel-plus digestion and distillation assembly: Di SWI-M, KPS-006R, Kjeldahl digestion unit and Kjelplus distillation unit (Pelican Instruments, Chennai, India) were used to estimate protein content in the samples.

Muffle furnace: The Muffle furnace (NSW pvt. Ltd, Delhi, India) maintained at 550° ± 10°C was used to estimate the ash content in the final product.

Spectrophotometer: Double beam 2023 Spectrophotometer was used for the determination of absorbance values of encapsulated powder and yoghurt samples to calculate the antioxidant activity.

Analytical reagents: Chemicals and reagents used in this study were of analytical grade and all aqueous solutions were made using glass distilled water.

3.2 PRELIMINARY TRIALS: (EXPERIMENTAL DESIGN):

Standardised milk (Fat 3%, SNF 10%) obtained was converted to yoghurt using method described by Tamime and Robinson, 1999. Yoghurt was prepared with different proportions 1-3% of whey protein isolate, whey protein concentrate and sodium caseinate based encapsulated resveratrol powder offered to semi trained panel of judges was for sensory evaluation on 9 point hedonic scale. On the basis of sensory scores of the product the optimization of the product was carried out.

Method of manufacturing:

Standardization of milk: Milk was standardized to (3% fat and 10% SNF) with whole milk and skim milk by Pearson Square method.

Heating of standardized Milk: heating of standardized milk to 85°C for 15 min.

Cooling of standardized milk: cool the standardized milk to 42°C.

Inoculation of culture: inoculation was done with *S. thermophilus* and *L. bulgaricus* @3% at 42°C. Incubation was done at 42°C for 4-5 hours.

Storage: storage was done at 5°C.

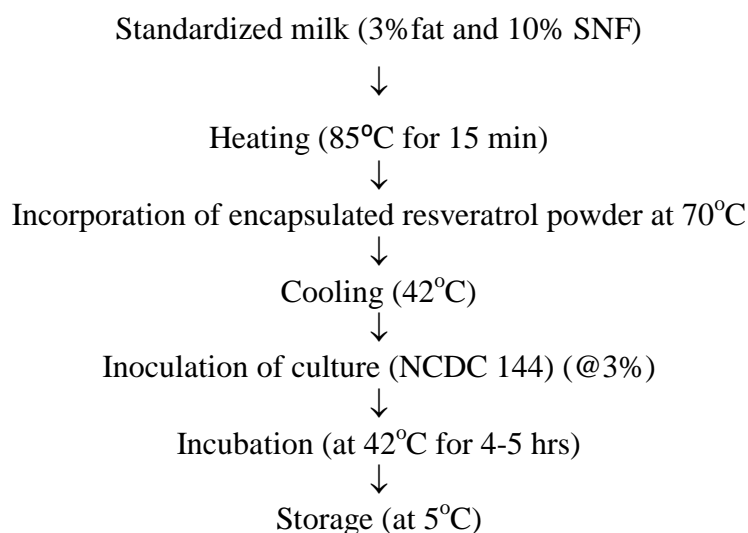


Fig 3.1: Flow Diagram of functional Yoghurt manufacture

3.3 Sensory Analysis

Seven panellists from College of Dairy Science and Technology, GADVASU, Ludhiana participated in this study. Prior to sensory evaluation, sensory panel was briefed about the desirable characteristics of the product. Nine-point hedonic scale was employed to carry out the evaluation of samples for sensory attributes such as colour and appearance, body and texture, flavour and overall acceptability (Amerine *et al* 1965). The nine-point hedonic scale includes various scales of grading i.e. liked extremely (9), liked very much (8), liked moderately (7), liked slightly (6), neither liked nor disliked (5), disliked slightly (4), disliked moderately (3), disliked very much (2), disliked extremely (1) as per the method described by the Lawless and Heymann 1998.

3.4 ANALYTICAL PROCEDURES:

The brief description of different methods used to examine physico-chemical properties of functional yoghurt is given below.

3.4.1 Physicochemical Analysis:

Moisture content: (Medler Toledo, USA) was used to analyse the moisture content of yoghurt and encapsulated powder samples. From the moisture content, total solids (100 – moisture %) was calculated.

Fat content

Fat was determined with the help of Mojonnier flask apparatus as specified in Indian standards (1981). Ten g sample of yoghurt was accurately weighed into a small beaker. Two ml of concentrated ammonia solution (sp.gr. 0.88) and 9 ml of distilled

water were added. The contents were swirled gently, cooled and with 10 ml ethyl alcohol (95% v/v), the contents were completely transferred to Mojonnier fat extraction tube and swirled for 2 minutes. Twenty five ml each of diethyl ether (sp. gr. 0.721; peroxide free) and petroleum ether (40°-60°C BP) were added and the tube was closed with a wet cork and shake vigorously for 1 minute. The tube was centrifuged for 1 minute to separate the ethereal layer from the aqueous layer and the supernatant liquid was decanted in clean, dry previously weighed aluminium dish containing glass beads. Extractions were repeated twice using 15 ml of diethyl and petroleum ether each time. The solvents were evaporated off and the dish heated in the oven at 98-100°C, cooled and weighed. Fat was calculated by weight difference method.

Protein content

Protein content was determined by Kjeldahl method as described by AOAC (2006). Two g of yoghurt sample was weighed accurately and transferred to a Kjeldahl digestion tube followed by addition of 2 g of digestion mixture comprising of K₂SO₄, CuSO₄ and SeO₂ (in ratio 100:10:1) and 15 ml concentrated H₂SO₄. The contents were then digested to a transparent clear fluid. The digested material was transferred to a 100 ml volumetric flask and distilled water was added to make the volume upto the mark. An aliquot (10 ml) of digest was distilled with 50% sodium hydroxide and the liberated ammonia was collected in 10 ml saturated boric acid containing 2-3 drops of mixed indicator (prepared by dissolving 100 mg methyl red and 30 mg methylene blue in 60 ml of 95% ethyl alcohol and then making up the volume to 100 ml with distilled water).

Approximately 65 to 70 ml of distillate was collected in a 100 ml conical flask. The contents of the flask were titrated against 0.02N HCl. A blank analysis using distilled water in place of sample was also carried out separately parallel to main sample. The total nitrogen and percent protein were calculated as follows:

$$\text{Per cent Nitrogen} = \frac{14.007(S-B) \times \text{Normality of HCl} \times \text{dilution factor} \times 100}{1000 \times \text{Weight of sample}}$$

where,

X = ml of HCl required for sample

Y = ml of HCl required for blank

N = Normality of HCl used, and

W = Weight of the sample in mg.

Percent Total Protein = Percent total nitrogen x 6.38

Titrateable acidity (% lactic acid)

The titrateable acidity was determined as per method of AOAC (2006). Ten g of sample was taken in a porcelain dish and mixed homogeneously by adding 10 ml hot distilled water (65°C). This was followed by addition of 1 ml of 0.5% phenolphthalein indicator. The mixture was titrated against 0.1N Sodium hydroxide with continuous stirring till the pink colour disappeared completely. Acidity was expressed as lactic acid/g of sample.

Syneresis

Syneresis of yoghurt samples were done by using the method as described by Hassan *et al.* (2015) with slight modifications. 25ml of set yoghurt at 5°C was slowly transferred to 50 ml capacity centrifuge tubes causing minimum disturbance to the coagulum. The centrifuge tubes were balanced by adjusting their weights and centrifuged at 339 rpm (Remi centrifuge Mumbai, India) for 20 min. The quantity of whey separated at the top of the coagulum inside centrifuge tubes was recorded as milliliters. The weight fraction of the supernatant liquid was used as index of whey syneresis (ml/100 g yoghurt).

Ash content

Ash content was determined by the procedure given by AOAC (2006). About 10 g of the product was weighed accurately in a silica dish and ignited on a bunsen burner with final incineration in a muffle furnace at 550±10°C for 2 hours. After incineration, it was allowed to cool by transferring the dishes to desiccator. After proper cooling, dishes contained ashed sample was weighed till the difference between the two consecutive weighings was less than 0.2 mg. Ash content was expressed as percent of the gross product.

Total carbohydrate content:

Proximal analysis was performed to estimate total carbohydrate content of functional yoghurt using the following formula:

$$\text{Carbohydrate \%} = 100\% - (\text{fat\%} + \text{protein\%} + \text{moisture\%} + \text{ash \%})$$

Where total fat, protein, moisture and ash contents were estimated using the respective analytical procedures described above.

Total calorific content (kcal) : The total calorie value of the sample was calculated

by summing up total fat content multiplied by 9 kcal/g fat; total protein content multiplied by 4 kcal/g protein and total carbohydrate content multiplied by 4 kcal/g carbohydrate (Gopalan *et al* 1989).

3.4.2 Texture Profile Analysis

Various textural properties such as firmness, consistency, cohesiveness and index of viscosity were measured using Texture Analyser TA-XT Plus (M/s Stable Micro Systems, Surrey, UK) equipped with 5 kg load cell. A back extrusion test using 40 mm cylinder probe was used for texture profile analysis of the yoghurt samples. The product was subjected to compressive force by probe up to the distance of 30 mm. For each evaluation, 3×3×2.5 cm³ size sample was used during texture analysis. Numerical values of textural values were measured using the exponent software (version 6.1.1.0). The conditions set in the Texture Analyser for measuring textural properties were as follows:

Pre-Test Speed, 1 mm/s;

Post-Test Speed, 1 mm/s;

Test speed, 1 mm/s;

Trigger force, 10.0 g;

Time: 5.0 s.

The following texture profile parameters were studied:

- **Firmness:** The peak force of the first compression cycle (first bite) (g).
- **Consistency;** area within curve during extrusion thrust (g.s)
- **Cohesiveness:** The ratio of positive force area during the second compression to that during the first compression (A_2/A_1) (g)
- **Index of viscosity;** area within negative region of curve during probe withdrawal (g.s).

3.4.3 Instrumental Colour measurement

Surface colour of yoghurt samples were measured using Colour Flex Colorimeter (Hunterlab, Reston, Virginia) supplied along with the universal software Easy Match QC (version 4.62) and the results were expressed in terms of CIE-LAB system. The colorimeter was equipped with dual beam xenon flash lamp as source of light. Prior to analysing the samples, the instrument was calibrated with standard

black glass and white tile as specified by the manufacturer of the equipment. Data was received through the software in terms of values for L* (lightness, 0 (black) to 100 (white)); a* (redness, +60 (red) to -60 (green)) and b* ((yellowness, +60 (yellow) to -60 (blue)) (Hunter 1975).

The sample of yoghurt was placed in the transmission port of the optical unit of a colour difference meter. Light energy from a controlled source was directed through the specimen into an integrating sphere. Phototubes are positioned at the top of the sphere to view the interior of the sphere. Electrical signals proportional to light quantities present are directed by cable to the measuring unit, where they are read directly as L*, a* and b* colour values.

3.4.4 Analysis of encapsulated resveratrol powder and yoghurt

Antioxidant Activity of powder and yoghurt

Radical scavenging activity of the encapsulated powder and yoghurt incorporated with encapsulated resveratrol was determined by DPPH (2, 2 diphenyl-1-picryl hydrazyl) method (Barkat *et al* 2013).

Resveratrol content in fortified yoghurt and encapsualted powder

- Reverse-Phase HPLC —Waters (USA)
- Column—C18
- Detector : UV detector (306nm) [Mobile phase—70(methanol):30(water)]
- Flow rate—0.8 ml/minute [Run time—10 minutes].
- For the detection of resveratrol, the optimal wavelength of 306 nm was used. Resveratrol concentrations was determined by measuring the peak areas and comparing them with the peak areas of known standard. Sample preparation was carried out as per Kim *et al.*, 2003 with modification.

Encapsulation Efficiency

Encapsulation efficiency was determined and calculated by the method of Bae and Lee (2008) with modifications.

3.4.5 MICROBIOLOGICAL STUDIES

Lactic acid bacteria, yeast and mold count and coliform count were recorded as per procedure by Wehr and Frank (2004) using the media De Man Rogosa and Sharpe (MRS) and M17 for Lactic acid bacteria, Potato dextrose agar for yeast and mold and MacConkey for coliform.

De Man Rogosa and Sharpe (MRS) broth and M-17 broth (HiMedia, India) were used for propagation of the *L. bulgaricus* and *S. thermophiles*, respectively.

Preparation of dilution blanks

The dilution blank consisted of 0.08% (w/v) sterile sodium chloride solution in 99 ml and 9 ml portions in screw capped dilution test tubes. These were autoclaved at a temperature (121°C) for 20 minutes (15 psi). The dilution blanks were warmed to 45°C before use for preparation of samples.

Preparations of dilutions

10 ml of yoghurt sample was drawn from the cups using a sterile pipette. The sample was then transferred to a sterile dilution blank of 99 ml. This gives a dilution of 1:100 from this initial dilution further dilutions were prepared by transferring 1 ml into 9 ml blank.

Lactic acid bacteria:

The count was enumerated using MRS and M17 agar. Media was rehydrated by dissolving 55.15 grms in 1000 ml distilled water for MRS. and for M17 the media was rehydrated by suspending 42.25 grms in 1000 ml distilled water. The mixture was then boiled to dissolve the media completely. It was then filled in conical flask and the mouth of the conical flask was closed with cotton plugs. The conical flasks were then sterilized by autoclaving at 15 psi pressure (121°C) for 15 minutes.

One ml of the diluted sample (suitable dilution) was transferred in each of the triplicate petri dishes. Then in each petri dish 15-20 ml of the melted agar (at 45°C) was poured, and the contents were mixed well by rotating in a clock wise and anti-clockwise position slowly. The contents were allowed to solidify at room temperature. The plates were then inverted and incubated at 37°C for 24-48 hrs.

Yeast and mould count

The count was enumerated using potato dextrose agar (PDA). Media was rehydrated by dissolving 39 g of dry media in 1000 ml distilled water. The mixture was then boiled to dissolve the media completely. It was then filled in conical flask and the mouth of the conical flask was closed with cotton plugs. The conical flasks were then sterilized by autoclaving at 15 psi pressure (121°C) for 15 minutes. One ml of 10% tartaric acid was added to lower the pH of the media to 3.5.

One ml of the diluted sample (suitable dilution) was transferred in each of the

triplicate petri dishes. Then in each petri dish 15-20 ml of the melted agar (at 45°C) was poured, and the contents were mixed well by rotating in a clock wise and anti-clockwise position slowly. The contents were allowed to solidify at room temperature. The plates were then inverted and incubated at 25±2°C for 3 to 4 days.

Coliform count

The count was enumerated using MacCkonkey agar. Media was rehydrated by dissolving 40.07 g of dry media powder in 1000 ml distilled water. The mixture was then boiled to dissolve the media completely and then cooled to 45 °C. It was then filled in conical flask and the mouth of the conical flask was closed with cotton plugs. The conical flasks were then sterilized by autoclaving at 15 psi pressure (121°C) for 15 minutes.

One ml of the diluted sample (1:100 dilution) was transferred in each of the triplicate petri dishes. Then in each petri dish 15-20 ml of the melted agar (at 45°C) was poured, and the contents were mixed well by rotating in a clock wise and anti-clockwise position slowly. The media was allowed to solidify and then incubated after inverting the plates at 37±1°C for 2 days.

3.5. STORAGE STUDY

The optimised yoghurts were packed in polystyrene cups and stored at 5°C for 15 days and the product was analysed for its changes in sensory parameters, physico-chemical, texture and microbiological at 3 days interval and the results are presented in following paragraph.

3.6. CONSUMER STUDY

To know the acceptability of developed product, final optimized sample of yoghurt was offered to perspective consumers (100) belonging to different age group. Consumer's response about the product was recorded through questionnaire (**Appendix-II**) supplied along with the samples to all consumer participated in consumer survey.

3.7 COST ESTIMATION

Cost of the yoghurt sample was calculated for one batch 100 kg by taking into considerations of all fixed and variable cost along with certain sets of assumptions made.

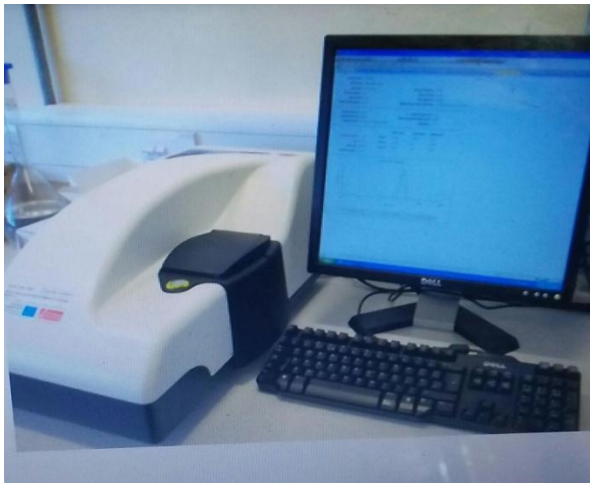
3.8 STATISTICAL ANALYSIS

The data of the samples obtained from various experiments were statistically analyzed by Analysis of Variance using statistical software SPSS 20.0 (IBM).for Windows Evaluation Version. A probability level of $P \leq 0.05$ was used for testing the statistical significance of all experimental data.



Ultrasonic Processor Vcx 750

Ultra Sonicator (Probe)



Particle Size Analyser



Spray Dryer



Control and Optimized Yoghurt in Incubator

CHAPTER IV

RESULTS AND DISCUSSION

Milk and milk products are widely consumed by all age groups and play a key role in nutrition throughout life. Yoghurt is regarded as a product of high moisture content and good quality proteins. The product is accepted by consumers because of its flavour and aroma components mainly acetaldehyde and texture.

The present study entitled “A study on effect of encapsulated resveratrol powder for enhancing the functionality of yoghurt” was conducted with a systematic approach to incorporate the encapsulated resveratrol powder for preparation of functional yoghurt to enhance the value addition of yoghurt. Results obtained during selection and standardization of the recipe, process/product optimization, sensory, physico-chemical, textural, colour, antioxidant activity, resveratrol content and microbiological attributes of the functional yoghurt packed under polystyrene cups stored at 5°C, cost analysis and pilot consumer study results are presented and discussed in this chapter. The graphical tools are used for the representation of data obtained during these studies.

4.1 Optimization of Operating Parameters of Spray Drying for Encapsulation of Resveratrol powder and Quality Characteristics of Encapsulated Resveratrol powder

Spray-drying is the commonly used drying technology in food industry due to its low cost and availability of equipment. The selection of wall material is the most challenging step in encapsulation of bioactive components by spray drying technique. It should have high solubility, good emulsifying, drying and film forming properties and protection from atmospheric oxygen and processing conditions to core material. Milk proteins are most commonly used for the encapsulation of bioactive components like resveratrol due to its superior emulsifying and antioxidative properties. The optimizing the spray drying process at lower temperatures or the right choice of wall materials is required to preserve the antioxidants after the spray drying process. An important step in the encapsulation process is the selection of wall materials depending on the active ingredient, the system in which it is going to be applied and the release mechanism. The present study was focussed on to study the stable

nanoemulsion with different formulations consisting of different coating materials like WPI, WPC and Sodium caseinate along with the combination of Maltodextrin powder used for encapsulation of resveratrol (core material) were subjected to spray drying viz operating parameters like three different inlet temperatures (150, 160, 170°C±1°C). Based on the preliminary trials, the inlet temperature 160±1°C was selected. Inlet air temperature is an important factor to be considered in any encapsulation system, in order to have a final product with good quality. The other parameters are outlet temperature is 75±1°C, Atomizer pressure 3 bar, feed pump rpm 30 and the capacity of the spray dryer 2-3 litre water evaporation/hr. The obtained encapsulated resveratrol powder was packed in an aluminium foil pack and stored at ambient temperature (25–30°C) until used for fortification.

The powder obtained was evaluated for the encapsulation efficiency. Based upon the encapsulation efficiency, equal proportion of different protein based coating materials WPI, WPC and Sodium caseinate with the combination of Maltodextrin powder with 1% resveratrol as a core material was selected and subjected to quality evaluation characteristics viz Antioxidant activity (%), Resveratrol content (mg/g), Encapsulation Efficiency (%), Moisture content (%), Total Solids and (%), Bulk density (g/ml).

The antioxidant activity of WPI, WPC and sodium caseinate based wall materials along with maltodextrin used for encapsulation of core materials resveratrol was 71.82, 63.52 and 72.56 percent, respectively. In addition to resveratrol, milk proteins having free-radical scavenging properties due to various amino acids such as cysteine, tyrosine, tryptophan, phenylalanine and histidine and free sulphhydryl groups. The encapsulation efficiency of the different formulations of the protein based wall materials (WPI/WPC/Sodium caseinate) was 95.35, 73.96 and 96.12 percent, respectively. Higher solid content resulted in higher encapsulation efficiency. Higher solid content resulted in higher emulsion viscosity, promoting faster drying and thus resulted in better encapsulation efficiency. Resveratrol content was in the range of 33.156 to 34.102 mg/g for different formulations. Bulk density (g/ml) of the different formulations were in between 0.387 to 0.443. The moisture content of different formulations of the protein based wall materials varied in the narrow range of 3.1 to 3.4 % (Sivakumar *et al* 2016 & 2017)

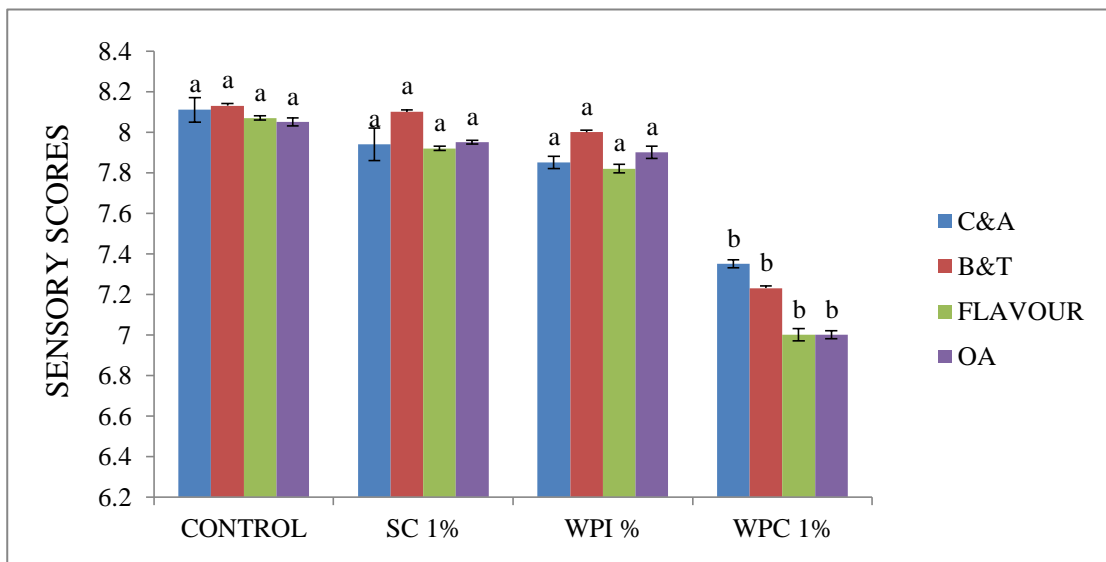
4.2 Preliminary Trials

Preliminary trials were conducted with broader ranges of different levels of milk protein based (WPI/WPC/SC) encapsulated resveratrol powder in yoghurt so that detailed overview of these variables can be studied. The levels for fat percent (3%) and SNF (10%) were kept constant for optimization of the product. The Fat and SNF contributes to enhancement in taste of the yoghurt with improved consistency and viscosity with reduced syneresis. It improves the flavour, rheological properties and over all acceptability of yoghurt (Guirguis *et al* 1984). During trials product was developed on the basis of variation of different levels of milk protein based (WPI/WPC/SC) encapsulated resveratrol powder. Broad range of ingredients and process parameters were determined based upon the results of sensory evaluation. The different ranges of ingredients were added during the development of product. Three levels of encapsulated resveratrol powder (1, 2 and 3%) were used during the development of product. Based upon sensory evaluation, 1% levels of all protein based encapsulated resveratrol powder were selected and kept constant in all yoghurt considering its role as an enhancer of functionality. More than above levels lead to bitter taste in the final product. The organoleptic quality of control and experimental yoghurt samples were evaluated by trained panellists selected from the faculty and students of College of Dairy Science and Technology, GADVASU, Ludhiana. The yoghurt samples (30-40g) were served with at 5°C and analysed for sensory parameters like colour and appearance, flavour, body and texture and overall acceptability using 9point hedonic scale. Sensory parameters play a key role in developing functional dairy products like yoghurt. These trials served as a basis for further optimization of the product.

4.3 Effect of encapsulated resveratrol powder on sensory parameters of yoghurt

The sensory scores for colour and appearance were 8.11, 7.94, and 7.85 for control, SC (1%), and WPI (1%), respectively. The score for WPC (1%) was 7.35. The difference in scores was due to low protein content of WPC as compared to SC and WPI. In case of the body and texture the scores obtained were 8.13, 8.09 and 8 for Control, SC (1%) and WPI (1%) respectively. The WPC (1%) score was 7.33, this is because of low protein content of whey protein concentrate, which gave yoghurt a loose body. Lower scores of body and texture scores may be due to lower gelling properties of whey proteins as compared to caseins. The score for flavour of yoghurts were 8.07, 7.92, and 7.82 for control, SC 1% and WPI 1% respectively.

Fig. 4.1: Effect of different encapsulated resveratrol powder on sensory characteristics of yoghurt



n=6

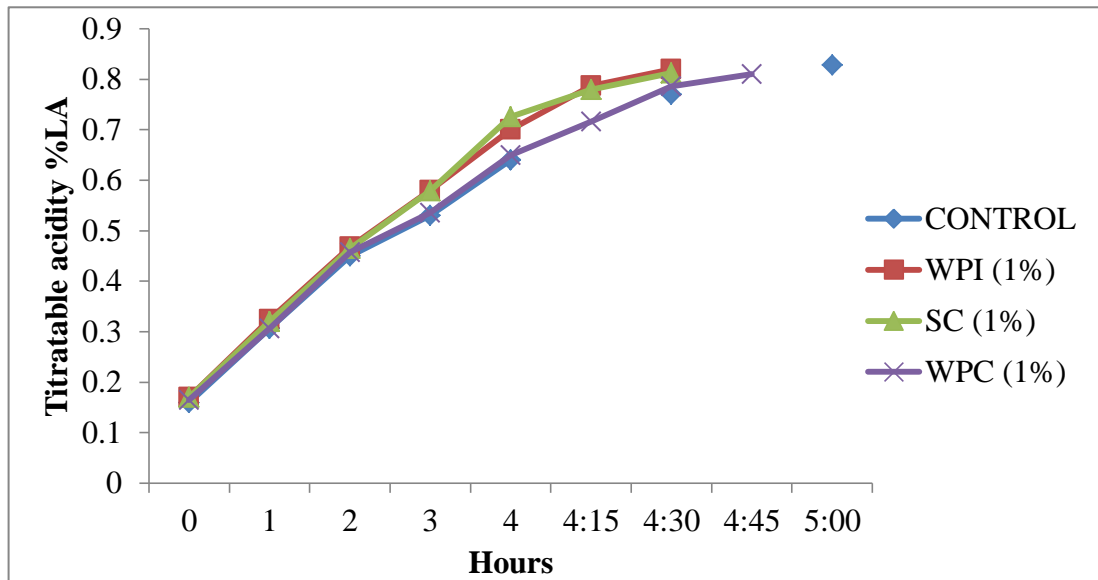
Mean with different superscript differs significantly ($p \leq 0.05$) and all the values are expressed as (mean \pm SE).

The score for WPC 1% was 7, because of low protein content and also low encapsulation efficiency which might release resveratrol content and led to bitter taste. The lower acceptability in terms of flavour could also be associated with the release of slightly higher amount of encapsulated resveratrol during pasteurization or fermentation process. Lower scores in yoghurt fortified with WPI based encapsulated materials as compared to SC based preparations might be due to lower gelling properties of whey proteins as compared to caseins. When tried with selected level of incorporation of different encapsulated powder, the entire sensory attribute like colour and appearance, body and texture, flavour and overall acceptability showed that sodium caseinate 1% and whey protein isolate 1% showed no significant difference ($p \leq 0.05$) as compared to control. The results are correlated with the findings of Goyal *et al* 2016. The scores for WPC 1% incorporated yoghurt was significantly ($p \leq 0.05$) different from other samples, the scores were lower than others led to unfit for further study. Finally, preparations at 1% level of encapsulated resveratrol powder (WPI/SC based) were selected for the development and physico-chemical analysis of fortified yoghurt.

4.4 Effect of fortification of encapsulated resveratrol powder on acidity and pH of yoghurt

Fermentation behaviour during different types of yoghurt preparation using encapsulated resveratrol powder

Fig. 4.2: Titratable acidity (% LA) of the samples after every hour during fermentation

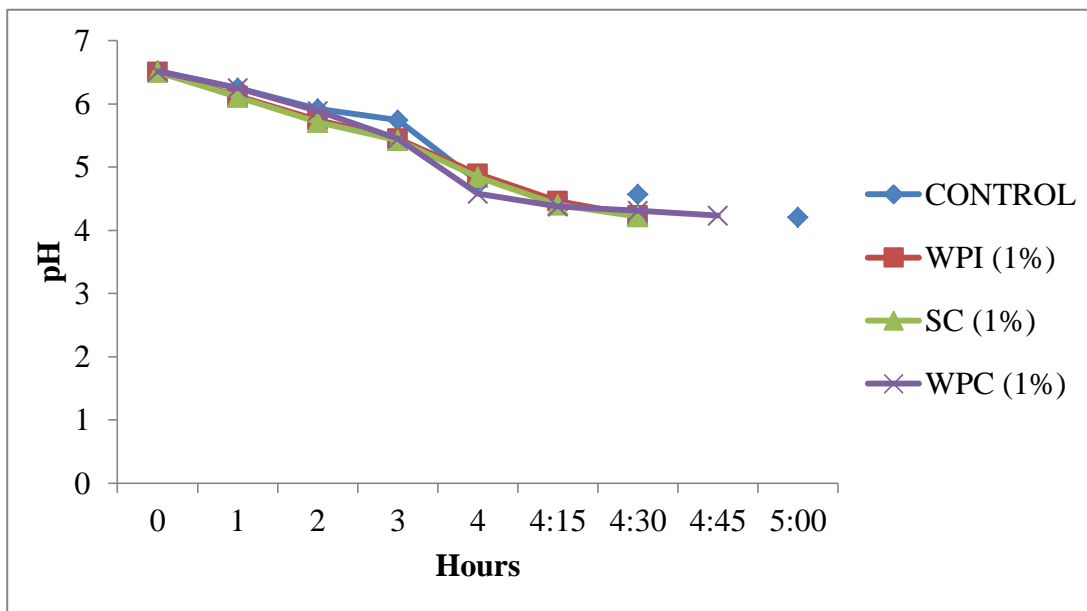


Mean values (n=6)

The acidity during the fermentation period was checked after every hour. The initial acidity of the milk before inoculation was 0.13% LA. The acidity in all the samples after addition of culture increased immediately and ranged between 0.16 to 0.175%. The use of high amount of inoculum (3%), higher total solids and protein content resulted in immediate increase in the acidity of the samples. At the end of 2 hours of incubation at $42\pm 1^{\circ}\text{C}$, the control sample showed an acidity of 0.45% LA. It was observed that the acidity of samples containing different wall materials (WPI/WPC/SC) used for encapsulation of resveratrol varied between 0.47 and 0.458 which were slightly higher than that in control sample. At the end of 5h of incubation, the control yoghurt has shown the acidity of 0.83 % which was ideal acidity for the yoghurt to be shifted to cold store (about 5°C). In case of WPC incorporated yoghurt, the acidity value was lower than WPI & SC based encapsulated powder fortified yoghurt because of low protein content. This showed that the rate of acid development in control and experimental samples significantly varied during the incubation period. The results findings are correlated with Lucey (2004) stated that an understanding of

the gelation process during fermentation is critical in manipulating the physical properties of yoghurt. The rheological properties of acid gels are affected by different starter concentration and incubation temperatures, which directly affect the rate of acidification.

Fig. 4.3: pH of the samples after every hour during fermentation



Mean values (n=6)

During incubation period of yoghurt pH was measured after every hour in control and fortified yoghurt sample. The initial pH of the milk used for yoghurt preparation was 6.64. At 0 hrs control yoghurt had 6.52 pH. But the yoghurt incorporated with encapsulated powder showed lower pH at 0 hrs, WPI 1% (6.504), SC 1% (6.5) and WPC 1% (6.514). After 2h of incubation, the control sample showed the pH of 5.92. On the other hand, encapsulated resveratrol incorporated yoghurt samples pH values between 5.71 and 5.89. Similar trend in pH change was observed at the end of 4h of incubation. After 4h of incubation period the control attained an optimum pH of 4.77. The control sample set at 5:00 hrs with pH value of 4.21, SC (1%) set at 4:30 hrs with 4.22 pH value, WPI (1%) set at 4:30 hrs with final 4.23 pH and WPC (1%) set at 4:45 hours with 4.235 pH. The different setting time and pH is due to high level of protein in encapsulated powder. But WPC 1% took more time because of lower protein content than other two powders. All the above pH values matched with the corresponding acidity values of the control and experimental yoghurt samples. Our results are correlated with the findings of increasing milk total

solids had significant effect on decreasing rate of pH during fermentation (Ozer et al 1998). The slow fermentation is not desirable for yoghurt because of typical increases in syneresis and a breakdown of symbiotic relationship between starter bacteria (Tamime and Robinson, 1999; Tamime et al 1989). The results were also supported by Ozturk and Oner (1999) who stated that after 4h of incubation at 42°C, the pH of the control was 4.26.

4.5 Proximate Composition of control and fortified yoghurt (Sodium caseinate and Whey Protein Isolate based wall materials used for encapsulation Resveratrol powder)

The milk was standardized to (3 % fat, 10% SNF) and yoghurt was prepared as per the standard procedure. The incorporation of encapsulated resveratrol powder contains different components like oil, resveratrol and addition of different protein based wall material (WPI/WPC/SC) with addition of maltodextrin significantly ($p \leq 0.05$) affected the proximate composition of the samples. The compositions varied as follows, fat (3.00 to 3.63%) protein (3.95 to 4.2%) carbohydrates, (5.25 to 5.62%) and ash (0.80 to 1.15%).

Table 4.1: Proximate Composition of control and optimized yoghurt

Parameter	Control	SC 1%	WPI 1%
Fat, %	3.00±0.05 ^c	3.63±0.00 ^a	3.56±0.01 ^b
Protein, %	3.95±0.00 ^c	4.17±0.00 ^b	4.2±0.00 ^a
Carbohydrates, %	5.25±0.01 ^c	5.57±0.01 ^b	5.62±0.01 ^a
Minerals, %	0.80±0.01 ^c	1.15±0.00 ^a	1.05±0.01 ^b
Total solids, %	13.00±0.03 ^c	14.52±0.00 ^a	14.41±0.00 ^b
Calorific value (Kcal/g)	63.8±0.01 ^c	71.63±0.01 ^a	71.32±0.01 ^b
Acidity (% LA)	0.85±0.00 ^c	0.88±0.00 ^a	0.86±0.00 ^b
pH	4.32±0.00 ^a	4.26±0.00 ^c	4.3±0.00 ^b
Syneresis (ml/100g)	2.61±0.01 ^a	2.1±0.02 ^c	2.31±0.01 ^b
Antioxidant Activity %	53.34±0.01 ^c	58.77±0.01 ^a	55.67±0.01 ^b
Resveratrol content (mg/100g)	NIL	35.156± 0.01 ^a	34.102± 0.00 ^b

n=6

Mean with different superscript differs significantly ($p \leq 0.05$) and all the values are expressed as (mean±SE).

In addition to the lactose from milk, carbohydrates from encapsulated components (Maltodextrin) have contributed for the total carbohydrates in the experimental samples. Normally milk contains about 0.80% minerals, while the control and experimental samples showed slightly higher minerals content, which could have been contributed by added encapsulated resveratrol components. The addition of culture, mixture of encapsulated resveratrol components has diluted the milk composition and resulted in variation of values in proximate composition of the experimental samples. The results were correlated with the findings of Sivakumar *et al* (2016).

The fresh control yoghurt showed the acidity value of 0.85% LA, while the corresponding value for experimental samples varied between 0.86 and 0.88% LA. Similarly the fresh control yoghurt showed the pH value of 4.32, while the corresponding value for experimental samples varied between 4.26 and 4.3. The fresh control yoghurt showed the syneresis of 2.61 ml per 100 g of the sample, while the corresponding values for experimental samples were in the range of 2.1 ml -2.31 ml per 100 gram of the samples. The syneresis in yogurt occurs when the casein particles rearrange themselves in the gel network leading to the spontaneous expulsion of whey without the application of any external force, otherwise it is defined as gel shrinkage that occurs concomitantly with liquid/whey expulsion and relates to the inability of the gel network to entrap all of the liquid phase (Serra *et al* 2009). It is dependent upon the total solids content, fat%, and the nature of proteins present in the product. The type of protein is different for all the samples. Higher syneresis in yoghurt samples added with control and WPI based preparations as compared to sodium caseinate yoghurt might be due to high whey protein to casein ratio, which induced the shrinkage of the gel, and subsequently increased whey separation.

The antioxidant activity of control and optimised yoghurt values varied in the range from 53.34 to 58.77%. The optimised yoghurt values were high due to incorporation of different milk protein based wall materials and resveratrol component. The results findings were correlated with Kim *et al* (2009) stated that the effects of different types of plant materials added as functional ingredients in yoghurt increased the antioxidant properties of it. The resveratrol content of control was nil and optimised yoghurt values were varied in the range from 34.1 to 35.2 mg/100g of product.

4.6 Texture, colour profile and microbiological parameters of control and resveratrol fortified Yoghurt

Textural parameters

Textural characteristics of set dairy products such as yogurt can be described in terms of firmness, consistency, cohesiveness and index of viscosity. Texture characteristics depend upon the composition, type and amount of fat, nature of proteins and total solids present in the product. In the present study, though the total solids content were kept similar in all the samples, but the nature of the protein varied in the fortification substances. The firmness of control and optimised yoghurt values varied in the range from 190.19 to 262.17 g. Results indicated that firmness was lowest in control and highest in the samples fortified with sodium caseinate based encapsulated resveratrol powder than WPI based encapsulated resveratrol powder. The texture values firmness, consistency, cohesiveness and index of viscosity differed significantly ($p \leq 0.05$) between control and optimised yoghurt.

Table 4.2: Texture, colour profile and microbiological parameters of control and optimised Yoghurt

Parameter	Control	SC 1%	WPI 1%
Texture Profile parameters of Yoghurt			
Firmness (g)	190.19±0.02 ^c	262.17±0.01 ^a	234.27±0.01 ^b
Consistency (g.sec)	3974.49±0.01 ^c	5058±0.01 ^a	4865±0.01 ^b
Cohesiveness (g)	-55.04±0.01 ^c	-99.56±0.02 ^a	-81.06±0.2 ^b
Index of Viscosity (g.sec)	-112.53±0.01 ^c	-161.67±0.01 ^a	-121.17±0.01 ^b
Colour Profile parameters of Yoghurt			
L	90.16±0.03 ^a	88.86±0.03 ^b	87.76±0.02 ^c
a	-1.73±0.02 ^a	-0.94±0.04 ^c	-1.17±0.02 ^b
b	9.88±0.01 ^c	10.39±0.04 ^b	11.46±0.03 ^a
Microbiological parameters of Yoghurt			
<i>L. bulgaricus</i> (log cfu/g)	6.6±0.00 ^c	6.92±0.00 ^a	6.84±0.00 ^b
<i>S. thermophilus</i> (log cfu/g)	6.88±0.00 ^c	7.09±0.00 ^a	6.94±0.00 ^b
Coliforms (cfu/g)	NIL	NIL	NIL
Yeast and Mold (cfu/g)	NIL	NIL	NIL

n=6

Mean with different superscript differs significantly ($p \leq 0.05$) and all the values are expressed as (mean±SE).

Colour Parameters

L* value is an estimation of food whiteness. Whiteness in fluid milk results from the presence of colloidal particles such as milk fat globules and casein micelles, capable of scattering light in the visible spectrum. Previous reports have shown that consumers have the highest appeal for fluid milks with visual properties characteristic of whole milk and the perception of milk whiteness has been demonstrated to have the most positive influence on increasing consumer appeal. The incorporation of encapsulated resveratrol powder decreased ($P \leq 0.05$) the value of L* of the milk, therefore, it presented a darkening effect. It probably due to the absorption of water by the fortificant, as L* value of foods is associated to the amount of free water in the surface of a product more than the complete product. The L value of control and optimised yoghurt values were varied in the range from 87.76 to 90.16. The L values are reduced whereas b values towards increasing trend. The b value of control and optimised yoghurt values were varied in the range from 9.88 to 11.46. This way, when fortificant was incorporated in yogurts, a* values towards less greenness and b* values more yellowness and a decrease in L* values less whiteness were observed. Overall, colour profile parameters L, a, b differed significantly ($P \leq 0.05$) between the control and optimised yoghurt.

Microbiological Parameters

In the control sample and optimised yoghurt the *L. bulgaricus* count varied in the range of 6.6 to 6.92 (log cfu/g) and *S. thermophilus* varied in the range of 6.88 to 7.09(log cfu/g). The optimised yoghurts were having more viable beneficial bacteria due to protein component present in the fortified substances provide the source of nutrient. The coliform and yeast and mould counts were nil indicated that control and optimised products were prepared in good hygiene practices.

4.7 Storage study of the optimized functional yoghurt

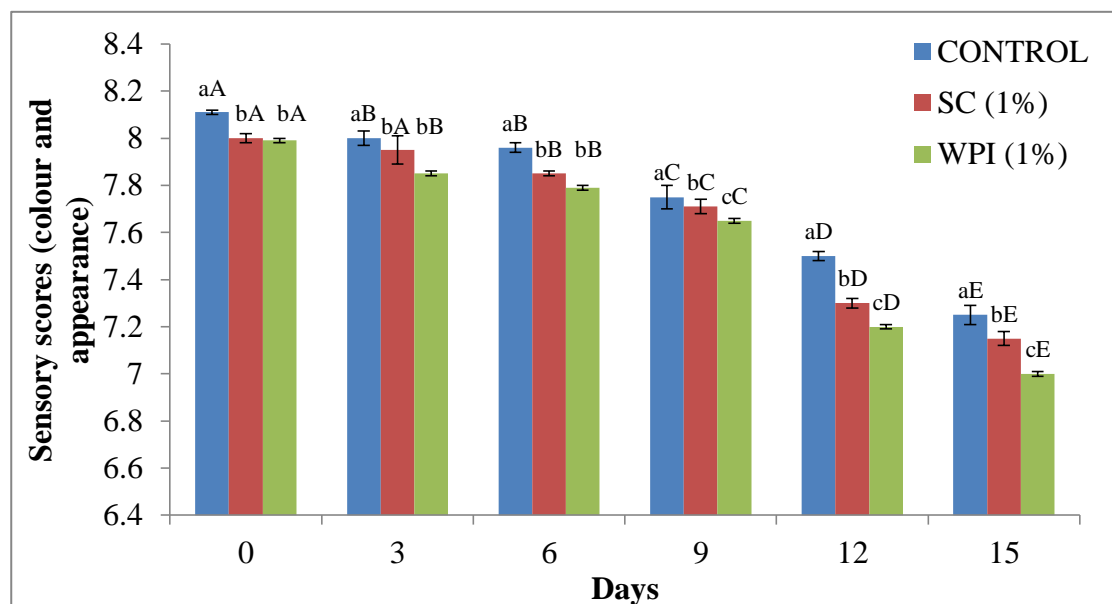
To evaluate the shelf life of functional yoghurt the product was stored at refrigeration ($4 \pm 1^\circ\text{C}$) temperature till the day of spoilage. The product was analysed for its sensory attributes, physico-chemical properties, textural, colour, antioxidant activity, resveratrol content and microbiological parameter at the regular interval at refrigeration ($4 \pm 1^\circ\text{C}$) temperature conditions. All the details are mentioned as follows.

4.7.1 Changes in sensory evaluation of functional yoghurt prepared by incorporation of encapsulated resveratrol powder during storage

Colour and Appearance

The colour and appearance scores presented in Figure 4.4 shows that the fresh control yoghurt scored 8.11, while SC based fortified yoghurt scored 8.00 and WPI based fortified yoghurt scored 7.99. For 0 and 3rd day the scores for SC based fortified yoghurt 1% shows non significant difference ($p \leq 0.05$) after that the scores reduced significantly because of slight expulsion of whey. For WPI based fortified yoghurt 1% the scores are decreased but showed no significant difference was observed on 3rd and 6th day after that the scores were reduced significantly. The scores were decreased because of the incorporation of encapsulated resveratrol powder and syneresis. At the end of 15th day control yoghurt scored 7.25, while SC based fortified yoghurt scored 7.15 and WPI based fortified yoghurt scored 6.99. During the storage period, the scores vary significantly within the samples and storage period. WPI got lowest score because of its color. The judges score was less due to development of dull appearance.

Fig. 4.4: Changes in colour and appearance of functional yoghurt prepared by incorporation of encapsulated resveratrol powder during storage



n=6

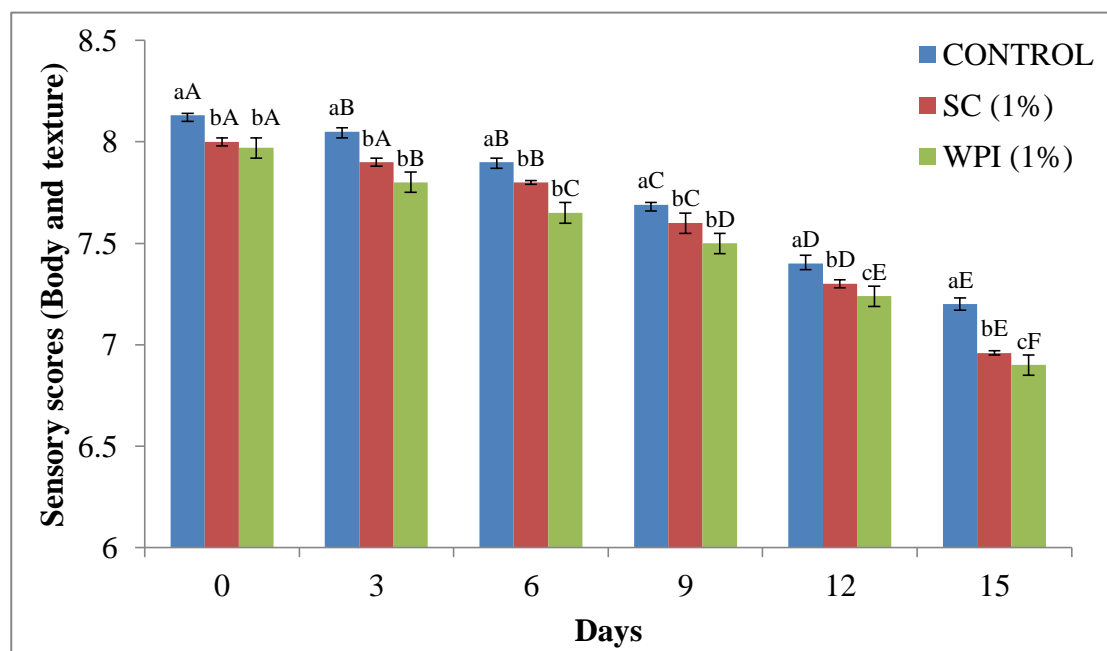
Mean with different superscript differs significantly ($p \leq 0.05$) and all the values are expressed as (mean \pm SE). Small letter shows difference between the samples, while capital letter shows difference between the days.

During storage, the appearance of yoghurt was affected and not acceptable which ultimately deteriorated the quality of yoghurt. It may be due to the growth of some yeast and mould in yoghurt during storage (Tarakci and Kuckoner 2003). The effect of storage between the samples (control and experimental yoghurt) was found significant for colour and appearance at ($P \leq 0.05$).

Body and Texture

The body and texture scores presented in Figure 4.5 shows that the fresh control yoghurt scored 8.13, while SC based fortified yoghurt scored 8.04 and WPI based fortified yoghurt scored 7.96. SC based fortified yoghurt 1% shows non significant difference ($p \leq 0.05$) during 0 and 3rd day but after that the scores were declined significantly. This is because at the starting the gel formation and bonding was high, but as the days increased because of the microbial action the gel loses its strength and showed loose body and syneresis. The optimized product (WPI/SC) showed significant difference ($p \leq 0.05$) on 12th and 15th day.

Fig. 4.5: Changes in body and texture of functional yoghurt prepared by incorporation of encapsulated resveratrol powder during storage



n=6
Mean with different superscript differs significantly ($p \leq 0.05$) and all the values are expressed as (mean \pm SE). Small letter shows difference between the samples, while capital letter shows difference between the days.

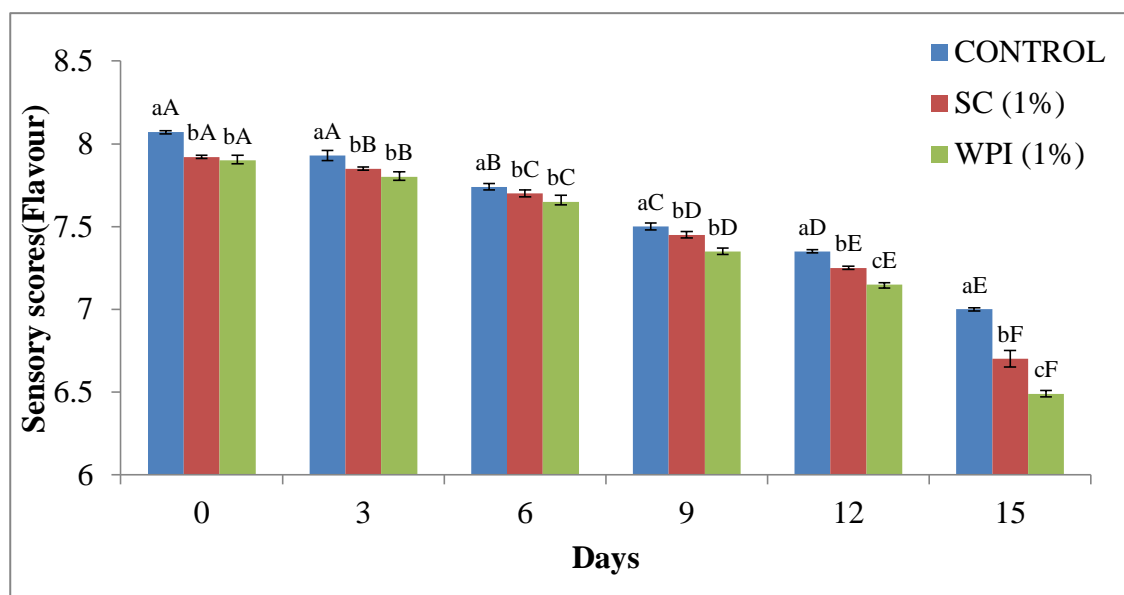
At the end of 15th day control yoghurt scored 7.2, while SC based fortified yoghurt scored 6.96 and WPI based fortified yoghurt scored 6.9. This may be because

of almost same protein content of SC and WPI. Over all, during storage the scores for body and texture are decreased significantly between storage period and samples.

Flavour

The flavour scores presented in Figure 4.6 shows that the fresh control yoghurt scored 8.07, while SC based fortified yoghurt scored 7.92 and WPI based fortified yoghurt scored 7.91. WPI 1% showed significant difference with control this may be because of release of some resveratrol content. At the end of 15th day control yoghurt scored 7.0, while SC based fortified yoghurt scored 6.60 and WPI based fortified yoghurt scored 6.49. Finally, at the end of the storage period the scores for the flavour of the control, SC based fortified yoghurt (1%) and WPI based fortified yoghurt (1%) decreased significantly ($p \leq 0.05$), this might be because of some amount of resveratrol may be released from encapsulated substances and also may be due to microbial effect. Abrahamsen (1978) reported that the decrease in flavour was due to the proteolytic activity of bacteria and the production of higher acidity in yoghurt. Loss of flavour can be attributed to fat and protein degradation (Mottar *et al* 1989).

Fig. 4.6: Changes in flavour of functional yoghurt prepared by incorporation of encapsulated resveratrol powder during storage



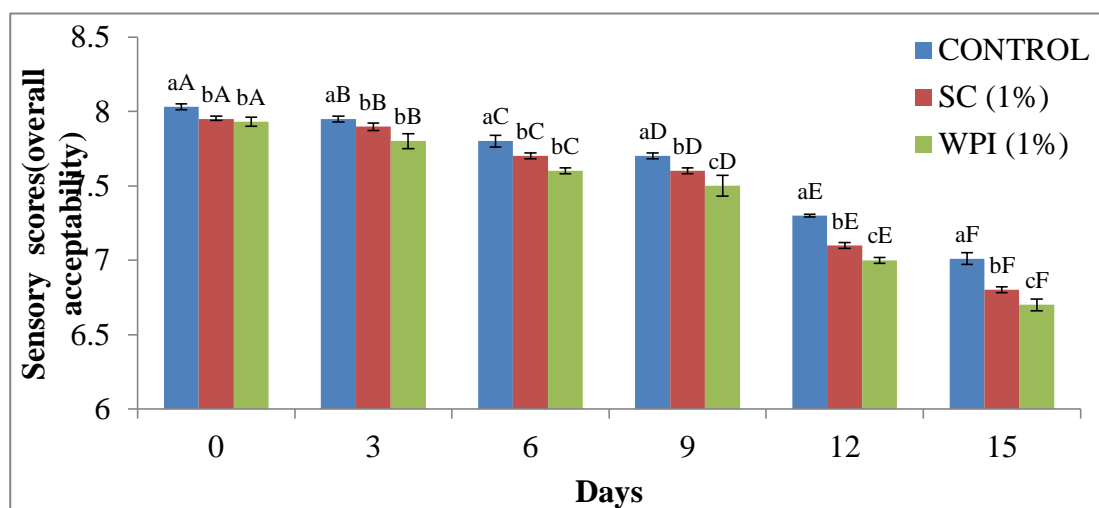
n=6

Mean with different superscript differs significantly ($p \leq 0.05$) and all the values are expressed as (mean \pm SE). Small letter shows difference between the samples, while capital letter shows difference between the days.

Overall acceptability

During the storage period, the overall acceptability of the samples decreases significantly ($p \leq 0.05$) because of syneresis, bitter flavour and loose body. The scores vary from 8.07 to 7 for control, 7.92 to 6.6 for SC based fortified yoghurt (1%) and 7.91 to 6.5 for WPI based fortified yoghurt 1%. This might be because of some amount of resveratrol content released from encapsulated components which gave bitter taste. Further, SC based fortified yoghurt (1%) showed less syneresis as compared to WPI based fortified yoghurt (1%) which affected overall acceptability of sensory scores. The overall acceptability is an overall evaluation influenced by colour and appearance, body and texture and flavour. The storage period showed significant effect on rheological and sensory characteristics of yoghurts. Mean scores for colour and appearance, body and texture, and flavour decreased significantly ($p \leq 0.05$) with increasing storage period. Spoilage of cultured dairy products is primarily caused by excessive fermentation, enzymatic breakdown or contamination with undesirable microorganisms. The low pH of yoghurt may offer a selective environment for growth of acid tolerant yeasts and moulds. Psychotropic organism may also pose a serious problem during storage at low temperature and result in defects like whey separation, ropiness, high acidity, gas formation and curdy body formation during storage. Overall, during storage the scores for overall acceptability were decreased significantly between storage period and samples.

Fig. 4.7: Changes in Overall acceptability of functional yoghurt prepared by incorporation of encapsulated resveratrol powder during storage



n=6

Mean with different superscript differs significantly ($p \leq 0.05$) and all the values are expressed as (mean \pm SE). Small letter shows difference between the samples, while capital letter shows difference between the days.

Changes in titratable acidity and pH of functional yoghurt prepared by incorporation of encapsulated resveratrol powder during storage

Titratable Acidity

During storage period acidity was increased and showed non significant difference ($p \leq 0.05$) for 0, 3rd and 6th day of storage. After that there was significant difference on control and optimized yoghurt samples. On 15th day the acidity of SC based fortified yoghurt (1%) was 1.09% LA and for WPI based fortified yoghurt (1%) level was 1.01% LA and for control yoghurt was 0.996 % LA. The results are in correlation with the findings of Andino (2011) who reported slightly higher acidity in omega-3 fortified yogurt prepared by adding microencapsulated salmon fish oil till 4 weeks of storage.

Table 4.3: Changes in Titratable acidity of functional yoghurt prepared by incorporation of encapsulated resveratrol powder during storage

Days	Control	SC 1%	WPI 1%
0	0.85±0.01 ^{cA}	0.88±0.02 ^{aA}	0.86±0.01 ^{bA}
3	0.86±0.02 ^{cA}	0.885±0.01 ^{aA}	0.871±0.01 ^{bA}
6	0.87±0.03 ^{cA}	0.89±0.01 ^{aA}	0.889±0.02 ^{bA}
9	0.911±0.01 ^{cB}	0.95±0.01 ^{aB}	0.93±0.01 ^{bB}
12	0.969±0.02 ^{cC}	0.991±0.02 ^{aC}	0.987±0.01 ^{bC}
15	0.996±0.01 ^{cD}	1.09±0.03 ^{aD}	1.01±0.01 ^{bD}

n=6

Mean with different superscript differs significantly ($p \leq 0.05$) and all the values are expressed as (mean±SE). Small letter shows difference between the samples while capital letter shows difference between the days.

The increase in total acidity which represents the amount of lactic acid present in the yoghurt during storage. In general, the total acidity increased during incubation, and this upward trend was almost linear compared to the pH change.

pH

Over all, during storage the pH value decreases as acidity increases there is significant difference ($p \leq 0.05$) between the samples and days. The pH reduction and increase in total acidity of supplemented yoghurts during storage could be possibly explained due to the higher consumption of residual lactose by the lactic acid bacteria.

Table 4.4: Changes in pH of functional yoghurt prepared by incorporation of encapsulated resveratrol powder during storage

Days	Control	SC 1%	WPI 1%
0	4.32±0.01 ^{aA}	4.26±0.00 ^{cA}	4.3±0.01 ^{bA}
3	4.3±0.00 ^{aA}	4.23±0.01 ^{cA}	4.28±0.01 ^{bA}
6	4.25±0.01 ^{ab}	4.21±0.00 ^{cB}	4.22±0.01 ^{bB}
9	4.21±0.00 ^{aC}	4.17±0.01 ^{cC}	4.2±0.01 ^{bB}
12	4.2±0.01 ^{aC}	4.15±0.01 ^{cC}	4.18±0.02 ^{bC}
15	4.15±0.00 ^{ad}	3.98±0.00 ^{cd}	4.02±0.01 ^{bD}

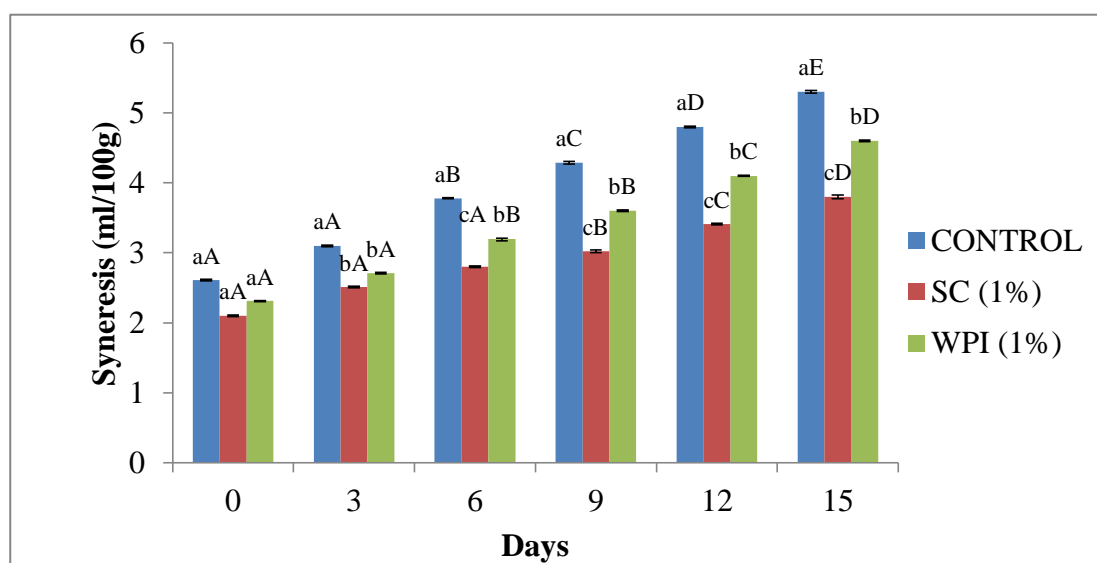
n=6

Mean with different superscript differs significantly ($p \leq 0.05$) and all the values are expressed as (mean±SE). Small letter shows difference between the samples while capital letter shows difference between the days.

Changes in Syneresis of functional yoghurt prepared by incorporation of encapsulated resveratrol powder during storage

Physical properties of yoghurt gels, including whey separation play an important role in quality and consumer acceptance. Syneresis is defined as gel shrinkage that occurs concomitantly with liquid/whey expulsion and relates to the inability of the gel network to entrap all of the liquid phase.

Fig. 4.8: Changes in syneresis of functional yoghurt prepared by incorporation of encapsulated resveratrol powder during storage



n=6

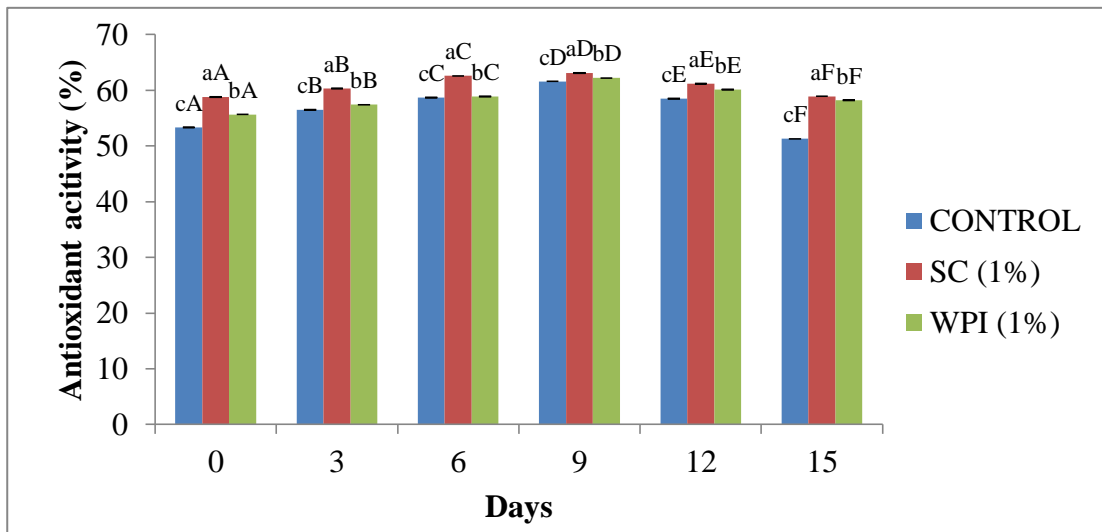
Mean with different superscript differs significantly ($p \leq 0.05$) and all the values are expressed as (mean±SE). Small letter shows difference between the samples, while capital letter shows difference between the days.

It was observed that syneresis increases throughout the storage period. SC based fortified yoghurt (1%) showed lowest syneresis as compared to others this may be because of high caseins content in sodium caseinate based yoghurt, as caseinate bind more water, so the expulsion of liquid decreases. There was significant difference ($p \leq 0.05$) between the control and optimized yoghurt during the storage period because of different water binding capacity. WPI based fortified yoghurt 1% shows high syneresis as compared to SC based fortified yoghurt 1% because of high whey protein to casein ratio. The results are correlation with the findings of Lucey (2004) and Farnsworth *et al* (2006).

Changes in Antioxidant activity of functional yoghurt prepared by incorporation of encapsulated resveratrol powder during storage

Yogurt is a rich source of bioactive peptides with antioxidant activity that are produced during fermentation. The optimized yoghurt showed more antioxidant activity than control this is because of the resveratrol content and protein content in the encapsulated powder. During storage the antioxidant activity increased for 9 days then decreased, this is because of the cultures consumes the protein content of the optimised yoghurt or this might be due to release of bioactive peptides during fermentation. The antioxidant activity showed significant difference ($p \leq 0.05$) between optimised and control yoghurt and also significant difference between the days. Our results were correlated with the findings of Korhonen and Pihlanto (2006) stated that milk proteins, the most important source of bioactive peptides and an increasing number of bioactive peptides have been identified in milk protein hydrolysates and fermented dairy products. Lactic acid bacteria used as starters are primarily responsible for the generation of bioactive peptides during milk fermentation (Nagpal *et al* 2011). Linh Nguyen and Eun-Sun Hwang (2016) stated that the higher antioxidant activity in aronia-containing yoghurt might result from the phyto chemical content of the juice and microbial metabolic activity. The DPPH radical scavenging activity of plain yoghurt was 59.47% this value was increased with aronia juice concentration incorporation.

Figure 4.9: Changes in antioxidant activity of functional yoghurt prepared by incorporation of encapsulated resveratrol powder during storage.



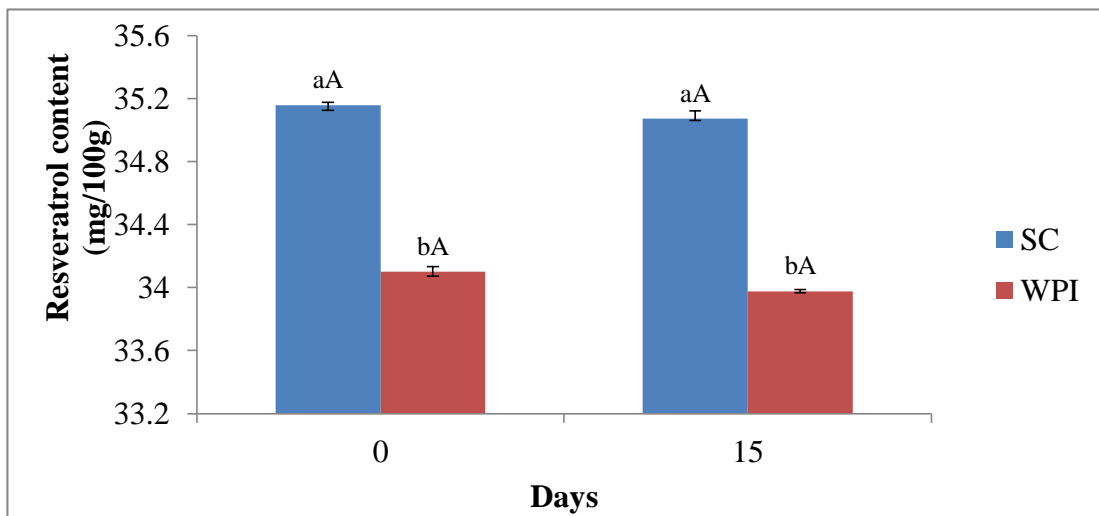
n=6

Mean with different superscript differs significantly ($p \leq 0.05$) and all the values are expressed as (mean \pm SE). Small letter shows difference between the samples, while capital letter shows difference between the days.

Changes in Resveratrol content of functional yoghurt prepared by incorporation of encapsulated resveratrol powder during storage

Resveratrol content (mg/100g) in yoghurt

Figure 4.10: Changes in Resveratrol content of functional yoghurt prepared by incorporation of encapsulated resveratrol powder during storage



n=6

Mean with different superscript differs significantly ($p \leq 0.05$) and all the values are expressed as (mean \pm SE). Small letter shows difference between the samples, while capital letter shows difference between the days.

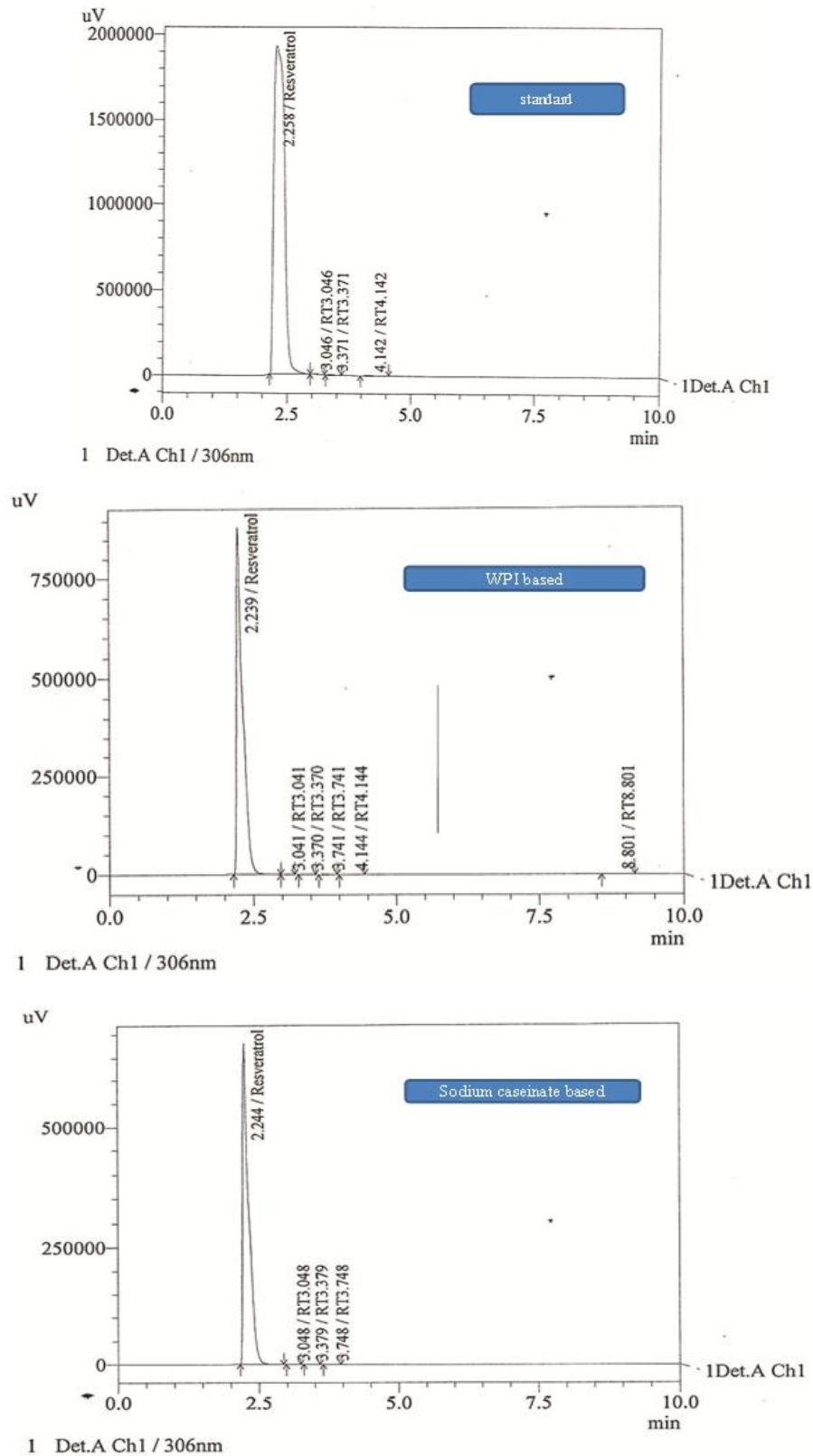


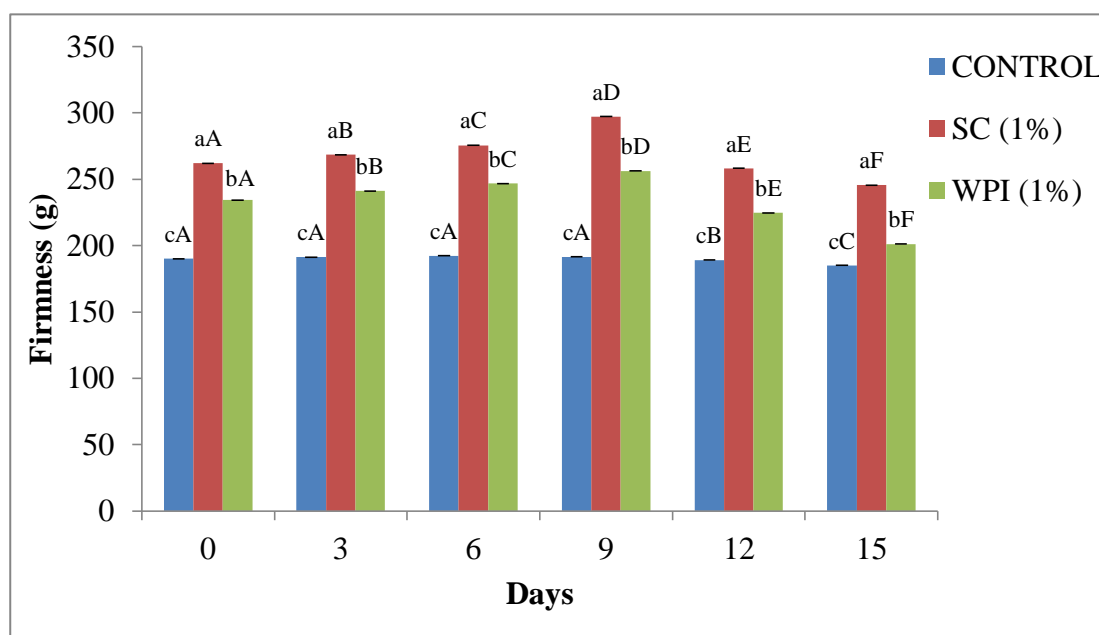
Fig. 4.11: HPLC Chromatogram of resveratrol standard and resveratrol content of functional yoghurt (Sodium caseinate based encapsulated powder and Whey protein isolate based encapsulated powder fortified yoghurt)

From the results, it was observed that resveratrol content of fresh (0 day) Sodium caseinate based encapsulated powder fortified yoghurt was 35.156 mg/100g and WPI based encapsulated powder fortified yoghurt was 34.102 mg/100 g . After 15th day of storage period, the resveratrol content of Sodium caseinate based encapsulated yoghurt was slightly reduced to 35.072 mg/100g and WPI based yoghurt was reduced to 33.976 mg/100g. However, significant decrease in resveratrol content was not observed during storage at 4°C.

Changes in Texture Profile parameters of functional yoghurt prepared by incorporation of encapsulated resveratrol powder during storage

Firmness depends on composition of yoghurt, type and amount of fat, nature of protein and total solids of yoghurt. With respect to firmness the control values are increased up to 6 days after that it started decreasing in trend. But at the same time optimised yoghurt the firmness values increased up to 9 days then it started decreasing trend up to 15 days, this may be due to proteolytic and lipolytic activities happening inside the yoghurt.

Fig. 4.12: Changes in firmness of functional yoghurt prepared by incorporation of encapsulated resveratrol powder during storage



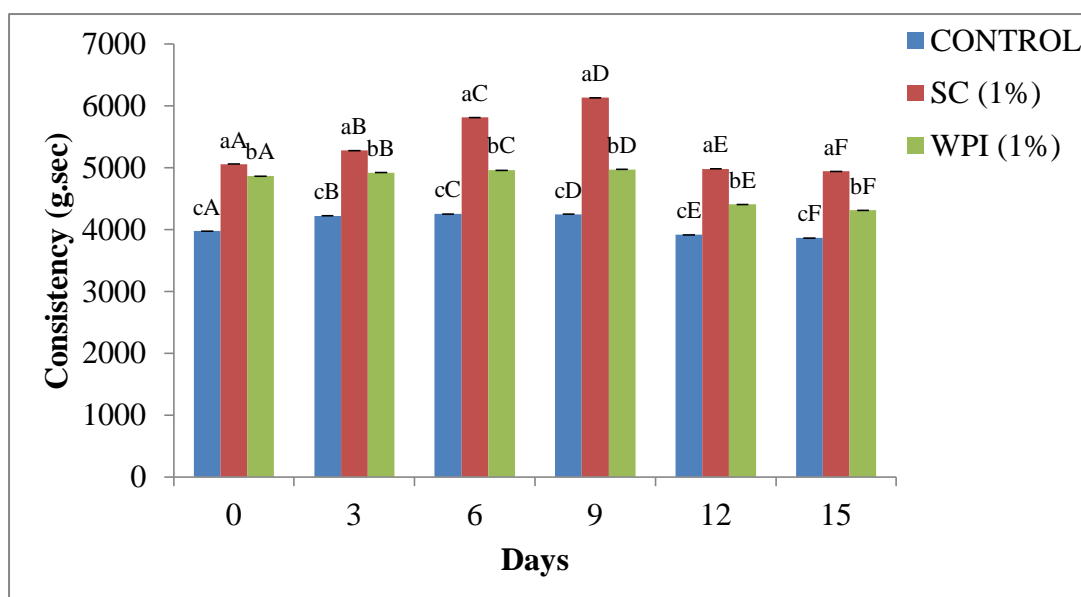
n=6

Mean with different superscript differs significantly ($p \leq 0.05$) and all the values are expressed as (mean \pm SE). small letter shows difference between the samples while capital letter shows difference between the days.

Comparatively higher firmness of sodium caseinate based yoghurt among all other samples could be attributed to the better gelling properties of casein micelles. There has a significant difference ($p \leq 0.05$) between the samples and storage period during storage study.

Gelation of milk proteins can be directly correlated with the hydration capacity. The ability of proteins to hydrate and entrap water without syneresis is different for caseins (4 g water per g protein) and whey proteins (0.3 to 0.6 g water per g protein). Casein micelles bind more water due to the enormous amount of void space within the casein micelles structure and binding of water to the hydrophilic surface of the micelles (Singh and Flanagan 2006). Caseinates provide better gelling properties and thus firmness in optimised yoghurt than that of whey proteins. Similarly, Akalin *et al* (2012) studied textural characteristics of probiotic yoghurts fortified with sodium calcium caseinate and WPC; and reported that fortification with sodium calcium caseinate increased the firmness more than WPC in probiotic yoghurts during storage ($p \leq 0.05$). Damin *et al* (2009) also showed that yoghurt made with milk supplemented with sodium caseinate resulted in significant increase in firmness.

Fig. 4.13: Changes in Consistency of functional yoghurt prepared by incorporation of encapsulated resveratrol powder during storage



n=6

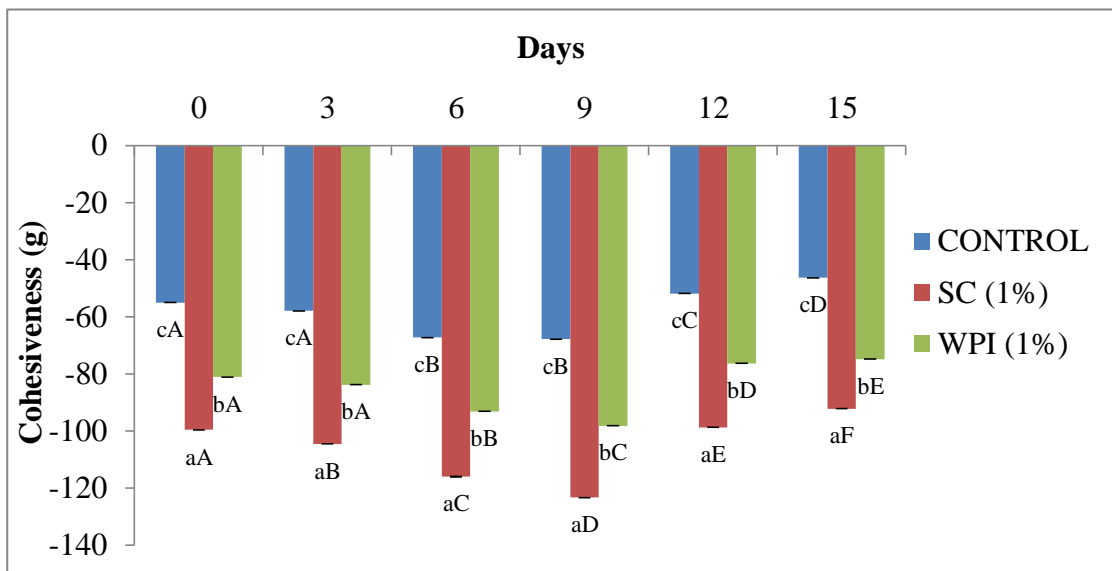
Mean with different superscript differs significantly ($p \leq 0.05$) and all the values are expressed as (mean \pm SE). small letter shows difference between the samples while capital letter shows difference between the days.

Consistency during the storage period first increased, then decreased this is because of good gelling capacity in the beginning days. After some time, due to proteolytic and lipolytic activities gel values decreased. There was significant difference ($p \leq 0.05$) within the samples and also storage period due to different total solids value of control and optimized yoghurt. Sodium caseinate based encapsulated powder fortified yoghurt shows highest consistency among the three samples. During the storage period the consistency of optimized yoghurt changes significantly ($p \leq 0.05$) with control, and is probably due to microbial action.

Cohesiveness (greater strength of internal bonds) may indicate large casein-casein linkages during stress application. There was significant difference ($p \leq 0.05$) between the samples and storage period, this may be because of different total solids, protein content of samples. Sodium caseinate based encapsulated powder fortified yoghurt shows highest Cohesiveness among the three samples due to high casein content.

During the storage study the index of viscosity increases for the 9 days then decreases. During the storage period there is significant difference ($p \leq 0.05$) between the samples. This is because of the different interaction or bonding of the protein networks, when the storage period increases microbial activities which affect the networks of protein. Our results are correlated with the findings of Mottar *et al* (1989) stated that the adsorption of heat denatured whey proteins on the casein micelle surface affects yoghurt texture. The proportion of β -lactoglobulin and α -lactalbumin bind to the casein depends on the heating process and heat intensity. The electron microscopy, results shows that filaments of denatured β -lactoglobulin develops on the micellar surface and offers a barrier to micelle fusion when milk was fermented, resulting in inferior yoghurt texture.

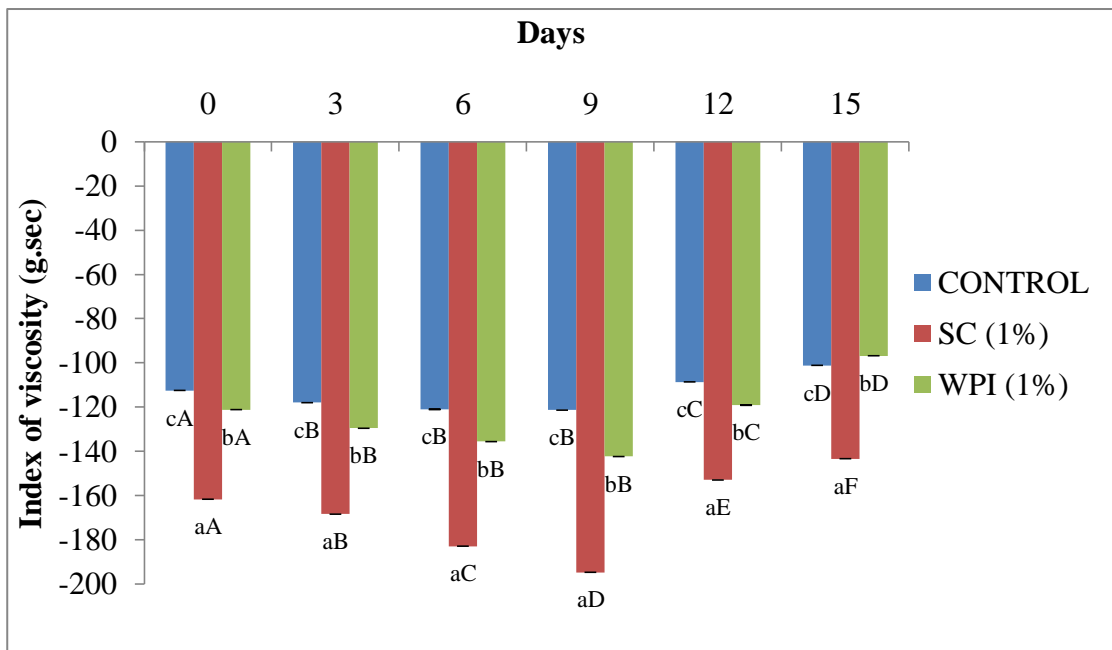
Fig. 4.14: Changes in Cohesiveness of functional yoghurt prepared by incorporation of encapsulated resveratrol powder during storage



n=6

Mean with different superscript differs significantly ($p \leq 0.05$) and all the values are expressed as (mean \pm SE). small letter shows difference between the samples while capital letter shows difference between the days.

Fig. 4.15: Changes in index of viscosity of functional yoghurt prepared by incorporation of encapsulated resveratrol powder during storage



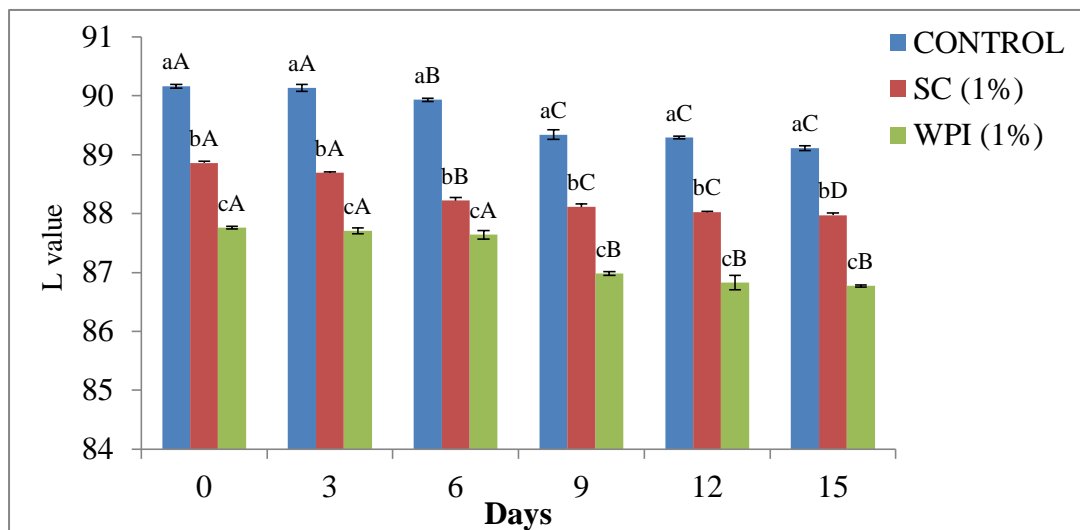
n=6

Mean with different superscript differs significantly ($p \leq 0.05$) and all the values are expressed as (mean \pm SE). small letter shows difference between the samples while capital letter shows difference between the days.

Changes in Colour Profile parameters of functional yoghurt prepared by incorporation of encapsulated resveratrol powder during storage

L value is lightness v/s darkness, where a low number (0-50) indicates dark and a high number (51-100) indicates light. L value of product is associated with amount of free water on the surface of product this way when powder is added to milk it is expected to absorb water thereby decrease in whiteness must occur. During storage the L value decreases significantly ($p \leq 0.05$) in control and optimised product. This may be because of whey expulsion. In between the samples and storage period also there was significant difference ($p \leq 0.05$) was observed. Control shows the highest value because powder was not incorporated in it.

Fig. 4.16: Changes in L value of functional yoghurt prepared by incorporation of encapsulated resveratrol powder during storage



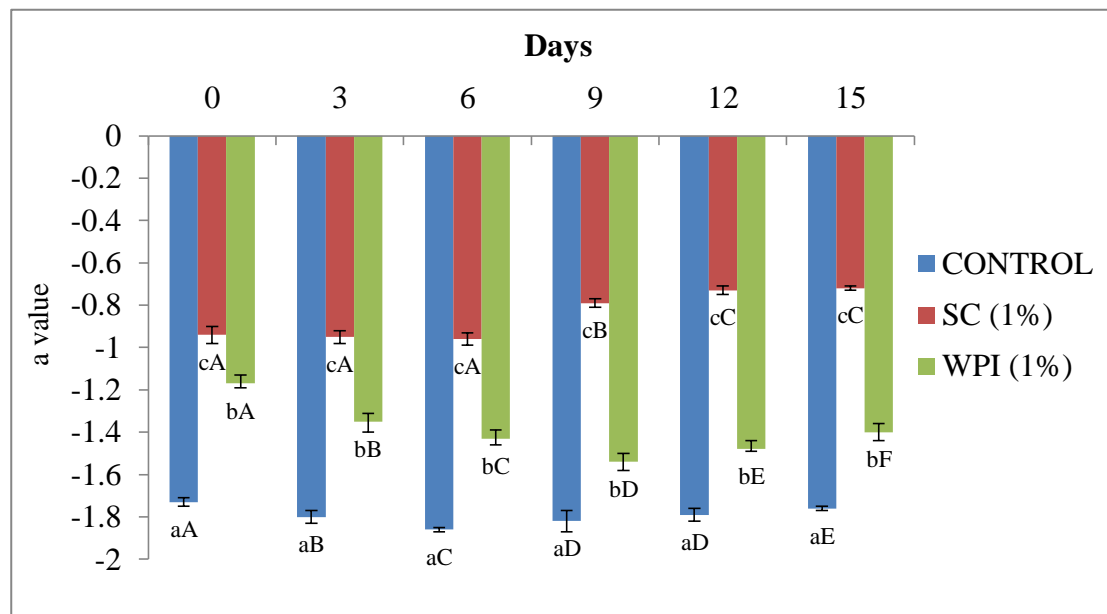
n=6

Mean with different superscript differs significantly ($p \leq 0.05$) and all the values are expressed as (mean \pm SE). small letter shows difference between the samples while capital letter shows difference between the days.

Colour value “a” showed redness v/s greenness, where a positive number indicates red and a negative number indicates green. Control shows the highest “a” value, WPI shows slightly low value. The SC caseinate showed lowest “a” value than other two. This may be related with syneresis of the samples and protein content of the fortificant, because the whey releases the gel contains riboflavin which have important green component. SC based fortified yoghurt 1% showing lowest syneresis as compared to others this may be because of high caseins content in sodium caseinate fortified yoghurt, as caseinate bind more water, so the expulsion of liquid decreases

due to this phenomenon the values are less in this optimized product. Between days and samples shows significant difference in the values ($p \leq 0.05$) during the storage period. These values are correlated with the findings of Shirai *et al* (1992).

Fig. 4.17: Changes in a value of functional yoghurt prepared by incorporation of encapsulated resveratrol powder during storage

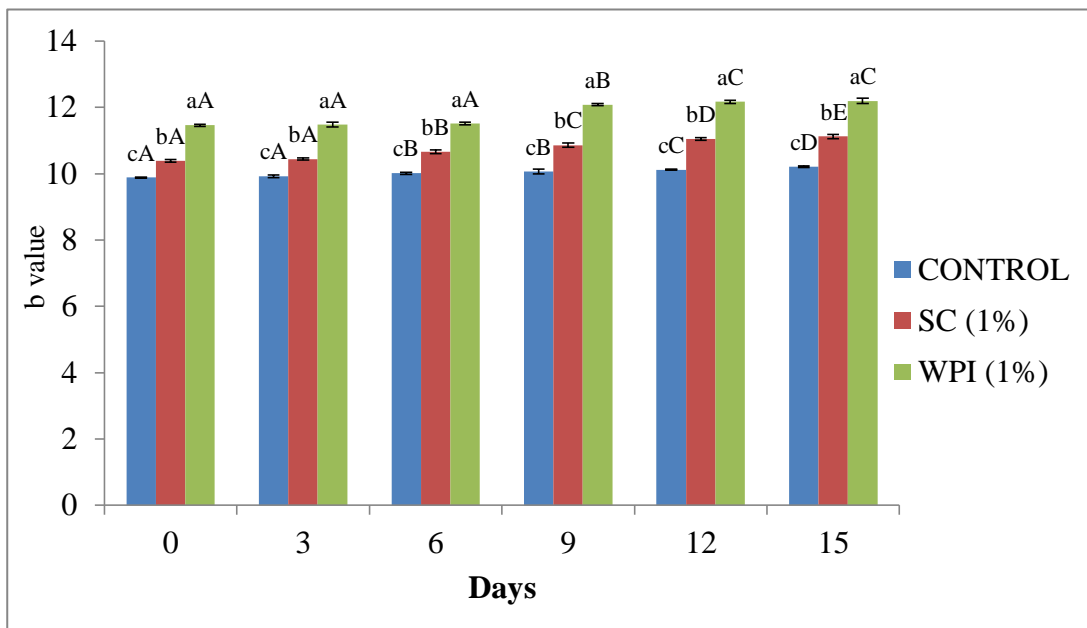


n=6

Mean with different superscript differs significantly ($p \leq 0.05$) and all the values are expressed as (mean \pm SE). small letter shows difference between the samples while capital letter shows difference between the days.

b Value indicates yellow v/s blue, where a positive number indicates yellow and negative indicates blue. During the storage period there was no significant difference ($p \leq 0.05$) of b value for the initial days between the optimised products, after that reduces significantly. Control showed no significant difference ($p \leq 0.05$) from 3rd to 9th day, after that reduced significantly. There was significant difference between the samples and days of storage. The results in correlation with the findings of Garcia-Perez *et al* (2005) and Hutchings (1994) who stated that the when the level of orange fiber percentage was increased in yoghurts, an increase of a* (less greenness) and b* values (more yellowness) and a decrease in L* values (less whiteness) were observed. This could be explained by the fact that once the yoghurt gets cold the fiber gets entrapped in the gel, impairing some of its color characteristics to the yogurt.

Fig. 4.18: Changes in b value of functional yoghurt prepared by incorporation of encapsulated resveratrol powder during storage



n=6

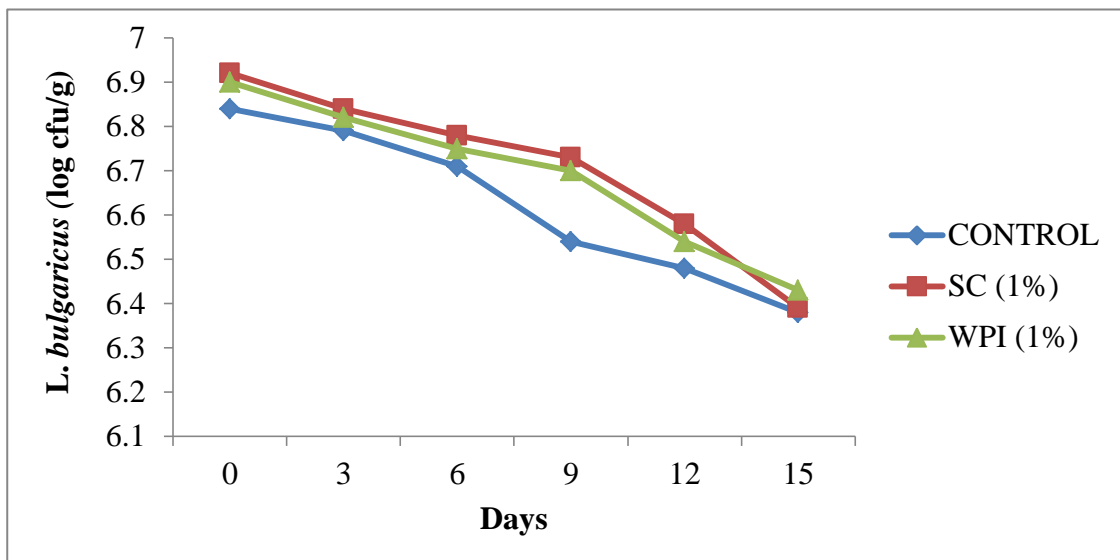
Mean with different superscript differed significantly ($p \leq 0.05$) and all the values are expressed as (mean \pm SE). small letter shows difference between the samples while capital letter shows difference between the days.

Changes in microbiological parameters of functional yoghurt prepared by incorporation of encapsulated resveratrol powder during storage

During storage, the *L. bulgaricus* counts decreased significantly ($p \leq 0.05$). On 0 day the counts for SC based optimised yoghurt and WPI based optimised yoghurt was 6.92 log cfu/gm and 6.9 log cfu/gm respectively and on the 3rd day the counts was reduced to 6.84 log cfu/gm and 6.82 log cfu/gm with a reduction of 0.08 log cfu/gm and 0.08 log cfu/gm respectively. On 15th day the reduction rate was about 0.63 log cfu/gm and 0.47 log cfu/gm respectively.

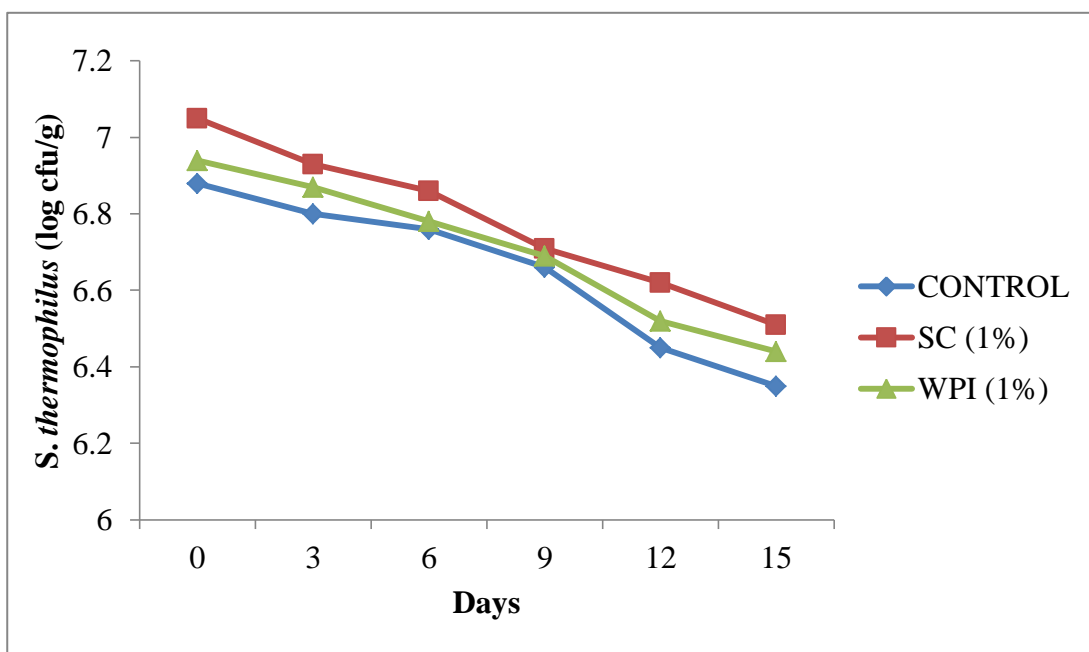
During storage, the *S. thermophilus* counts are significantly ($p \leq 0.05$) reduced. On 0th day SC based optimised yoghurt and WPI based optimised yoghurt shows 7.05 log cfu/gm and 6.94 log cfu /gm respectively 3rd day shows 6.93 and 6.87 log cfu/gm, with a reduction of 0.12 log cfu/gm and 0.07 log cfu/gm. On 15th day the reduction was 0.53 and 0.5 log cfu/gm, respectively in SC based and WPI based functional yoghurt.

Fig. 4.19: Changes in *L. bulgaricus* counts of functional yoghurt prepared by incorporation of encapsulated resveratrol powder during storage



Survival, as expected higher in refrigerated samples, and there was a relation between the decrease of pH and the decrease of bacterial counts. The high acidity is the main reason for the lower survival of lactic acid bacteria in fermented products with very low pH values. Also there was significant difference within the sample with respect to pH and acidity, because of different composition of yoghurt. Therefore the present findings are in correlation with the findings of Joon *et al* (2018).

Fig. 4.20: Changes in *S. thermophilus* count of functional yoghurt prepared by incorporation of encapsulated resveratrol powder during storage



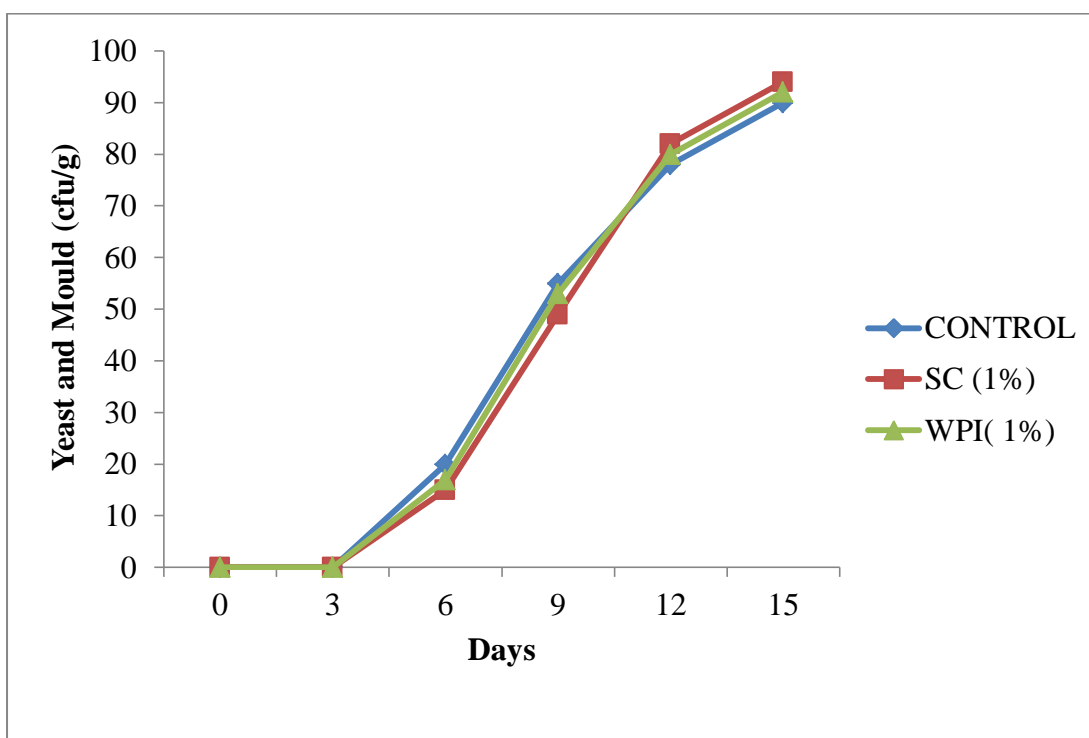
Coliforms

During storage, the coliforms count was zero, indicating good hygiene practices followed during product manufacture.

Yeast and Mould count

During storage conditions, upto third day there was no growth of yeast and mould was observed. From 6th day onwards slight growth of yeast and mould was detected. The counts varied significantly ($p \leq 0.05$) during storage. On 3rd day 15 cfu/gm was observed for SC based optimised yoghurt and 17 cfu/gm was observed for WPI based optimised yoghurt. On 15th day the growth was 95 cfu/gm for SC and 92 cfu/gm for SC. The counts increased significantly during storage and also showed significant difference among the sample. The growth was observed may be because of air born contamination. Upto 15th days the growth was less than the prescribed standards of FSSAI , therefore the product is safe to consume.

Fig. 4.21: Changes in yeast and mould count of functional yoghurt prepared by incorporation of encapsulated resveratrol powder during storage

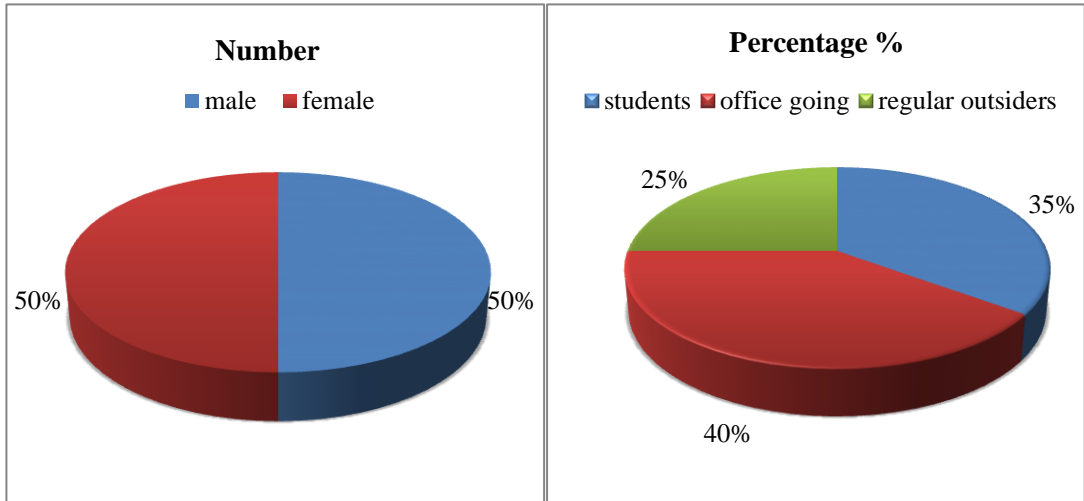


The microbiological analysis of the yoghurt samples during storage revealed that control yoghurt and optimised yoghurt was acceptable up to 15th days. After this period, coli form and yeast & mould counts were increased beyond the prescribed

limit of FSSAI standards. The microbial counts correlated with the sensory scores values.

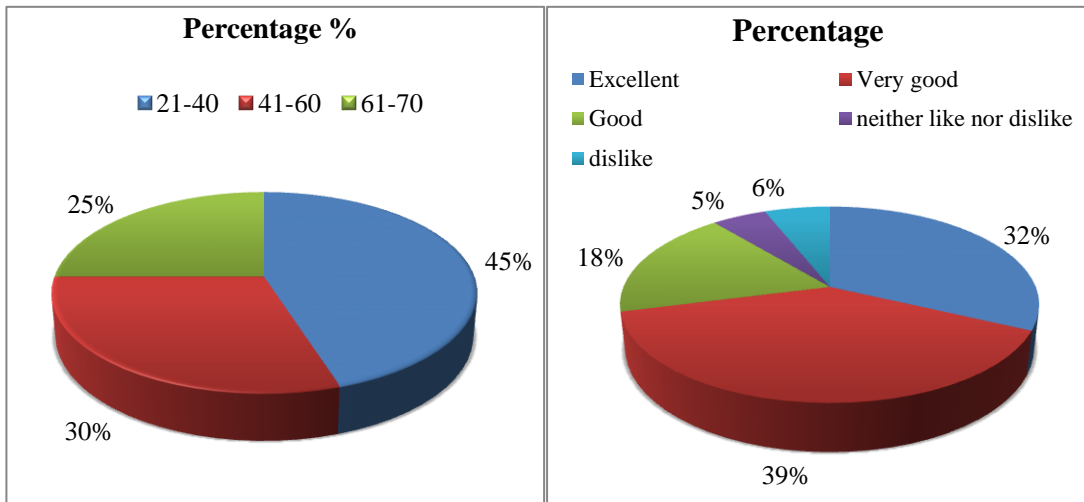
4.8 Pilot Consumer study of Yoghurt with Encapsulated resveratrol powder

Pilot consumer study on the acceptability of yoghurt sample was carried out to find out the potential marketability of the product. Yoghurt was optimised as per the earlier mentioned procedures and was packed in polystyrene cups (100 gm) and then distributed to 100 persons who were regular consumers of yoghurt. Among the consumers, nearly 50% were male and 50% were female. The breakup of the consumers presented shows that among the consumers nearly 35% were students 40% were office going people and remaining 25% regular outsiders. Age group distribution indicates that 45% of the consumers were in the age group of 21-40 years, 30% were in 41-60 years group and 25% were in 61-70 years group. The degree of liking among the respondents which is presented in Fig 4.2 shows that nearly 32% described the product as excellent and 39% as very good, while remaining 18% expressed the product as good. 5% of the people neither like or nor dislike the product and 6% of the people dislike the product. The following details given the representation of how the people consumed the product, 20% of people (alternate day) and 53% people (weekly basis), 17% (fortnightly), 6% (rarely) and 4% (never). Altogether the product was well accepted by the consumers. Therefore the present investigation revealed that optimised yoghurt was well accepted by the consumers.



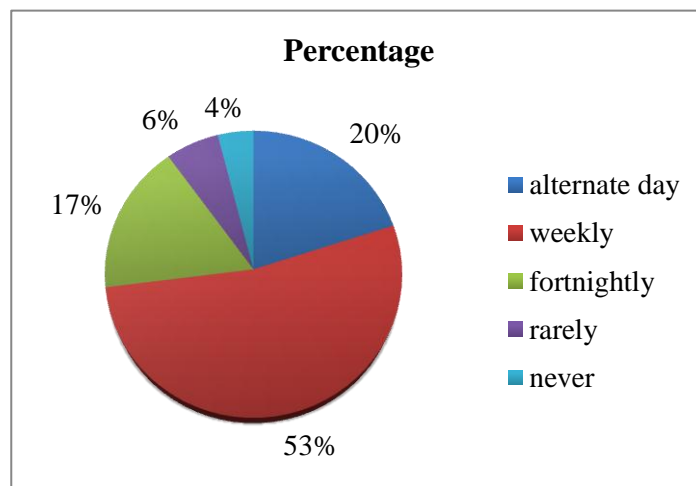
Consumer- Gender wise

Details of consumers



Age of the respondents

Degree of liking among Respondents



How often consumer take the product

Fig. 4.22: Pilot consumer study of the optimised product

4.9 Costing of the developed product

Yoghurt products have achieved considerable economic importance worldwide owing to their high nutritional image. Economic viability is the most important factor to take place in the market for any food product along with its quality and sensory parameters. Further, the product should also be attractive in terms of palatability and price for commercial sustainability of the product in a competitive market. Every market product needs to be commercially viable to become attractive for entrepreneurs and industrialists so that its mass production can be taken up. Therefore, through present investigation, an attempt was made to estimate the cost of production of optimized yoghurt considering certain set of assumptions:

1. Land and building

The working space required for optimized yoghurt unit was 900 sq. feet (30X30 sq. feet) and it was taken on rent (@ ₹10000/- per month) semi urban city of Ludhiana. Building includes processing section, store and utilities section.

2. Machinery and Equipment

Capital investment (₹10,00,000/-) includes the cost of all equipments viz. pasteurizing vat & its accessories, yoghurt cup filling and sealing machine(manual), incubator room, cream separator, refrigerator, walk in cooler, trays, Generator and accessories. Depreciation on machinery and equipments was taken @10 percent per annum. Interest on capital investment and variable cost was taken @12 percent per annum.

3. Raw material

Raw materials such as mixed milk, resveratrol, sodium caseinate, SMP, culture and other required ingredients were required for preparation of optimized yoghurt.

4. Capacity

One batch of optimized yoghurt (100 kg) was made from raw materials

5. Manpower

Two skilled and three unskilled people were required for manufacturing functional yoghurt and 300 working days of the year.

6. Packaging Material

The optimised yoghurts were packed in polystyrene cups and stored at 5°C@

Table 4.5: Component wise cost analysis

	Total Capital investment required	Rs. 10,00,000	
Sr No.	Particulars	Cost (in Rs.)	
A.	Fixed cost		
	Depreciation on Equipments @10% p.a.	100000	
	Interest on Capital Investment @12% p.a.	120000	
	Building rent per annum	120000	
	Fixed cost per annum	340000	
	Total fixed cost per day (A)	932	
B.	Variable cost (Rs/unit)	Quantity required per batch (100 kg)	Cost (in Rs.)
	Raw materials		
	a. Mixed milk for yoghurt preparation(26/kg)	94Kg	2444
	b. Skim milk powder(250/kg)	2 kg	500
	c. Culture(300/kg)	3Kg	900
	d. Encapsulated Resvertrol Powder	1Kg	2200
	Total		6044
	Labour @ 302/ Day for unskilled and 367/ Day for skilled (1 man day= 8 Hr)	Unskilled= 3 Skilled = 2	1941.8
	Packaging Material	1000	1000
	Electricity @ 8 / unit	40	320
	Laboratory charges@ 0.2% of raw materials		12.00(round ed)
	Cleaning and sanitizing materials@ 0.1% of raw materials		6.00(rounde d)
	Total variable cost per day (per batch) (B)		Rs. 9022
	Total cost of the batch = A+B	Rs. 9952	
C.	Returns from batch		
C	Yield per batch	100 Kg	
	Total cost per kg(9952/100kg)	Rs.99.52	
D.	Sale price per kg	Rs. 120	
	Profit per kg (Before taxes)	Rs. 20.48	
	Gross returns	12000	
	Total cost (Fixed +Variable)	9952	
	Net returns	2048	
	BC ratio (Gross returns/Total cost)	1.21	
	Breakeven point	31.28	
	Margin of safety (per cent)	68.72	

Break-even analysis

Break Even Analysis = Total fixed cost \ Sales per unit-Variable cost per unit

The break even output is the minimum number of units of finished product produced at which the total revenue equals total cost. A firm will continue its production process or will remain solvent as long as the marginal revenue is greater than or equal to the marginal cost. Break even output provides us with an estimate of the output produced at that level. The Break Even output was calculated as 31.28 kg of product.

Components wise cost classification

Proportion of fixed cost – 9%

Proportion of variable cost – 91%

Components of variable cost

It is evident from the graph that the largest share among the variable cost is of raw materials (67.00 %) followed by the Labour (18.00%) and miscellaneous includes, packaging material and other cost components like electricity, laboratory, cleaning and sanitizing together accounted for about 15% .

Final cost of production of optimised yoghurt was worked out to be ₹99.52 per kg . Considering the prevailing market price of yoghurt is ₹120 per kg, a profit of ₹20.48 per kg could be obtained which is a good profit margin for the commercial viability and sustainability of the product.

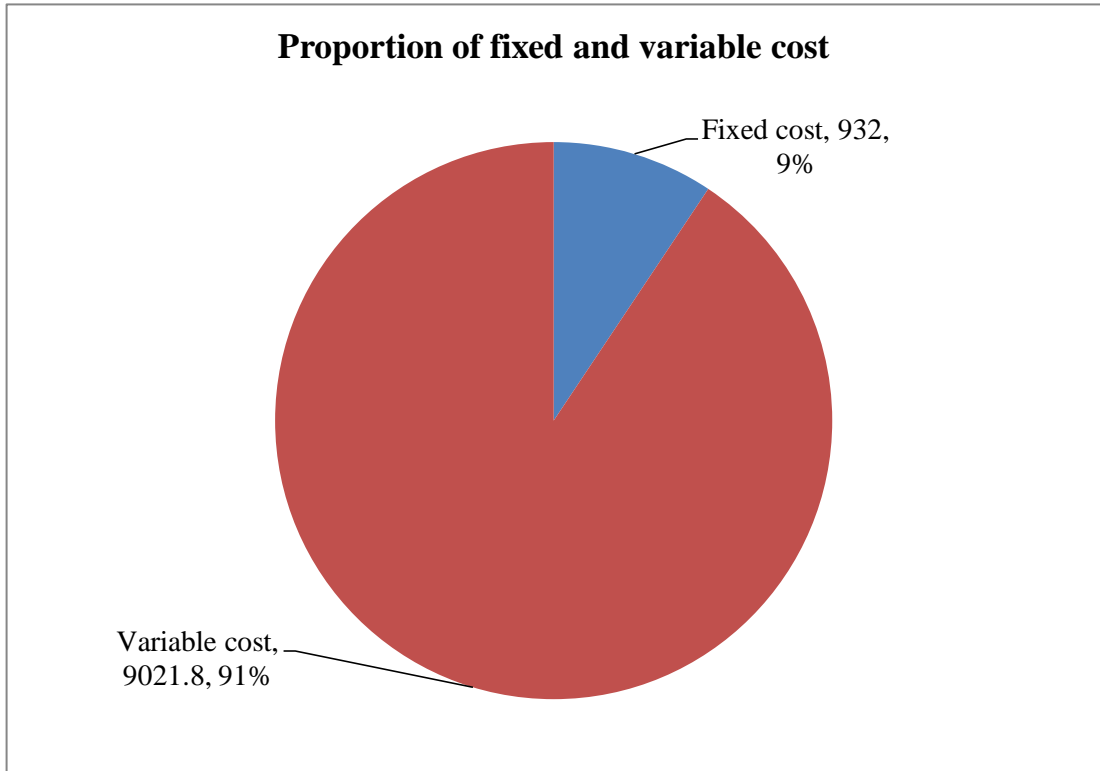


Figure 4.23: Proportion of variable and fixed cost

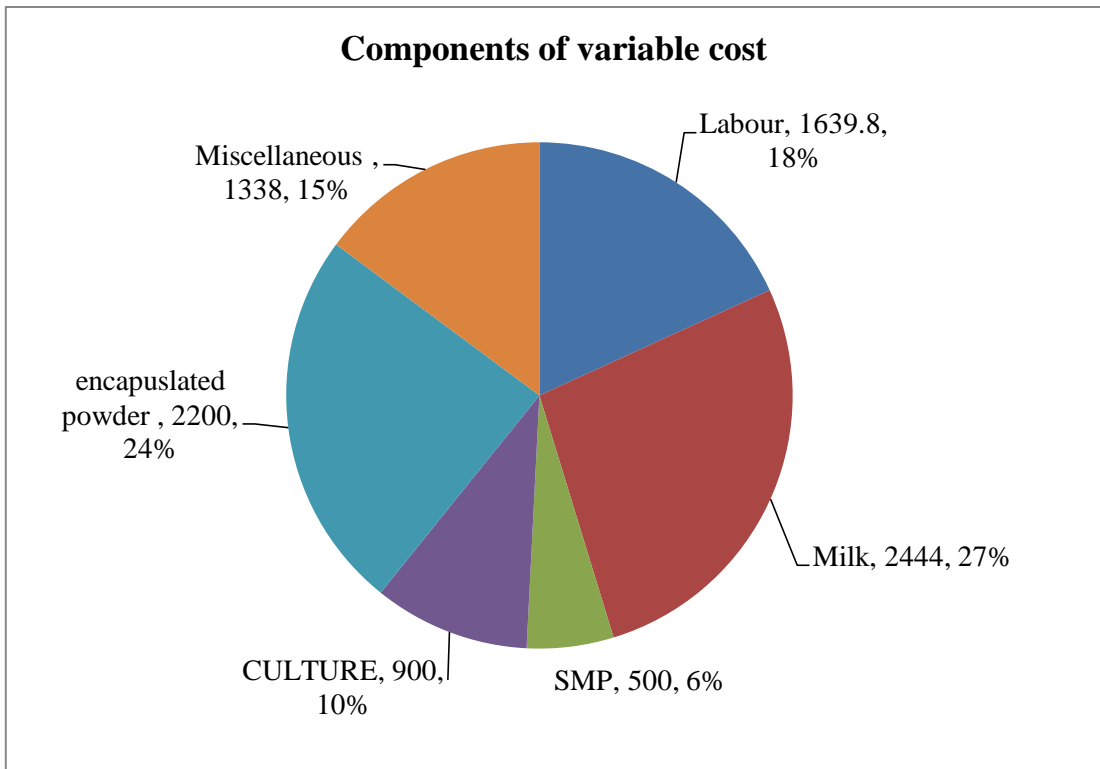


Figure 4.24: Components of variable cost

CHAPTER V

SUMMARY AND CONCLUSIONS

Fermented milk products are recognized by nutritionists as beneficial to human health, they are easily digestible and have high nutritional value. Yoghurt, the most popular fermented milk is a dairy product usually blended with functional ingredients is the target of our study. The present study entitled “A study on effect of encapsulated resveratrol powder for enhancing the functionality of yoghurt” was conducted with a systematic approach to incorporate the encapsulated resveratrol powder for preparation of functional yoghurt to enhance the value addition of yoghurt. The details are as follows:

5.1 Optimization of ingredients for preparation of functional Yoghurt

During preliminary trials product was developed on the basis of variation of different levels of milk protein based (WPI/WPC/SC) encapsulated resveratrol powder. Broad range of ingredients and process parameters were determined based upon the results of sensory evaluation. The different ranges of Ingredients were added during the development of product. Three levels of encapsulated resveratrol powder (0, 1, 2 and 3%), were used during the development of product. Based upon sensory evaluation 1% levels of all protein based encapsulated resveratrol powder were selected and kept constant in all yoghurt considering its role as an enhancer of functionality. The scores for WPC 1% incorporated yoghurt was significantly ($p \leq 0.05$) different from other samples, the scores were lower than others leads to unfit for further study. Finally, preparations at 1 % level of encapsulated resveratrol powder (WPI/SC based) were selected for the development and physico-chemical analysis of fortified yoghurt.

5.2 Proximate composition of control and fortified yoghurt

The incorporation of encapsulated resveratrol powder contains different components like oil, resveratrol and addition of different protein based wall material (WPI/WPC/Sodium caseinate) with addition of maltodextrin significantly ($p \leq 0.05$) affected the proximate composition of the samples. The compositions varied as follows, fat (3.00 to 3.63%), protein (3.95 to 4.2%), carbohydrates (5.25 to 5.62%), ash (0.80 to 1.15%). The antioxidant activity of control and optimised yoghurt values

were varied in the range from 53.34 to 58.77%. The antioxidant activity of optimised yoghurt values were high due to incorporation of different milk protein based wall materials and resveratrol component. The resveratrol content of control and optimised yoghurt values were varied in the range from 34.102 to 35.156 mg/100g of product.

5.3 Storage study of control and fortified yoghurt under refrigeration temperature (4±1 °C)

To evaluate the shelf life of product functional yoghurt the product was stored at refrigeration (4±1°C) temperature till its spoilage. The product was analysed for its sensory attributes, physico-chemical properties, textural, colour, antioxidant activity, resveratrol content and microbiological parameter at the regular interval of 3 days at refrigeration (4±1°C) temperature conditions. All the details are mentioned as follows

During storage all the sensory attributes viz., colour and appearance, texture, flavour, and overall acceptability decreased in refrigeration temperature.

During storage period acidity increases and shows no significant difference ($p \leq 0.05$) on 0, 3rd and 6th day of storage. After that there is significant difference on control and optimized yoghurt samples. On 15th day the acidity of SC based fortified yoghurt (1%) was 1.09 % LA and for WPI based fortified yoghurt (1%) level was 1.01% LA and for control yoghurt was 0.996. The increase in total acidity, which represents the amount of lactic acid present in the yoghurt during storage. In general, the total acidity increased during incubation, and this upward trend was almost linear compared to the pH change. SC based fortified yoghurt (1%) showing lowest syneresis as compared to others this may be because of high caseins content in sodium caseinate based fortified yoghurt, as caseinate bind more water, so the expulsion of liquid decreases. There was significant difference ($p \leq 0.05$) between the control and optimized yoghurt during the storage period because of different water binding capacity.

The optimised yoghurt show more antioxidant activity than control this is because of the resveratrol content and protein content in the encapsulated powder. The antioxidant activity shows significant difference ($p \leq 0.05$) between optimised and control yoghurt and also significant difference between the days.

From the results, it was observed that resveratrol content of fresh (0 day) sodium caseinate based fortified yoghurt was 35.156 (mg/100g) and WPI based

encapsulated fortified powder was 34.102 (mg/100 g). After 15 days storage period, the values for Sodium caseinate based encapsulated powder fortified yoghurt are slightly reduced to 35.072(mg/100g) and WPI based encapsulated fortified powder was reduced to 33.976 (mg/100g), this minor value change indicates, the fermentation behaviour didn't have any significant effect on the level of resveratrol during storage at 4°C.

Firmness depends on composition of yoghurt, type and amount of fat, nature of protein and total solids of yoghurt. Comparatively higher firmness of Sodium caseinate based encapsulated powder fortified yoghurt among all other samples could be attributed to the better gelling properties of casein micelles. Firmness values interlinked with other texture values, so there is a significant difference ($p \leq 0.05$) between the samples and days during storage study. The colour values also L, a and b values were difference ($p \leq 0.05$) between the samples and days during storage study.

The microbiological analysis of the yoghurt samples during storage revealed that control yoghurt and experimental sample was acceptable up to 15 days. After this period, coliform and yeast & mould counts were increased beyond the prescribed limit of FSSAI standards. The microbial counts correlated with the sensory scores of the product.

It has been discussed in previous sections that yoghurt samples fortified with resveratrol powder (SC based) significantly ($p \leq 0.05$) showed less syneresis and higher firmness than WPI based fortified yoghurt measured by instrument: objective evaluation; and also in subjective evaluation, there was no significant ($p \leq 0.05$) difference in the sensory scores of control and resveratrol fortified yoghurt samples, when evaluated on the same day of analysis. However, data showed that during the storage, sensory scores differed significantly ($p \leq 0.05$) in control as well as Resveratrol fortified yoghurt samples. It was concluded that on the basis physicochemical characterization novel variety of functional yoghurt was manufactured with incorporation 1% of sodium caseinate based encapsulated resveratrol powder. This product will boost the dairy industry and also helpful to the health conscious consumers.

5.4 Cost analysis and pilot consumer study of the final optimized product

Cost of production of the developed product was calculated for a batch size of 100 Kg. The total fixed and variable costs were calculated. The average cost per kg of the final product was calculated to be ₹99.52/- which was quite low as compared to the prevailing market price for yoghurt leading to good profits making the product commercially viable. By taking the prevailing market price of yoghurt a profit of ₹ 20.48 could be earned which indicates that the product is having good potential for commercial success and viability. The break even output was calculated at 31.28 kg of product.

Finally the developed product is acceptable upto 15 days of storage at refrigeration temperature as well as commercial viability. Therefore it is safe to conclude that the product is having good scope for commercialization and entrepreneurship development.

The degree of liking among the consumer respondents shows that nearly 32% described the product as excellent and 39% as very good, while remaining 18% expressed the product as good. 5% of the people neither like or nor dislike the product and 6% of the people dislike the product. Altogether the product was well accepted by the consumers. Therefore the present investigation revealed that optimised yoghurt was well accepted by the consumers.

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APPENDIX – I

**COLLEGE OF DAIRY SCIENCE AND TECHNOLOGY
GADVASU (LUDHIANA)**

Score card for sensory evaluation of resveratrol fortified yoghurt

Product particulars: RESVERATROL FORTIFIED YOGHURT Date: _____

Kindly evaluate the given samples of *resveratrol fortified yoghurt* for flavor, body& texture, colour and appearance and overall acceptability using the following 9-point hedonic scale and enter the score for each sample in the space provided in the below table.

Hedonic ratings	Score
Liked Extremely	9
Liked Very Much	8
Liked Moderately	7
Liked Slightly	6
Neither Liked nor Disliked	5
Disliked Slightly	4
Disliked Moderately	3
Disliked Very Much	2
Disliked Extremely	1

Sensory Attributes	Sample Code				
Flavor					
Body& Texture					
Color& Appearance					
Overall Acceptability					

Remarks (if any): _____

Signature: _____

APPENDIX – II

COLLEGE OF DAIRY SCIENCE AND TECHNOLOGY

GADVASU (LUDHIANA)

Performa for Pilot Consumer Study of Developed Product

1	Name	
2	Age	
3	Occupation	
4	Address	
5	Degree of liking among respondents	Excellent Very Good Good
6	How often you take the product	Alternate day Weekly Fortnightly Rarely Never
Date:		Signature:

VITA

Name of the student : Harshita Sonarhi
Father's name : Shri. Vishnu Lal Salvi
Mother's name : Smt. Geeta Salvi
Nationality : Indian
Date of birth : 19-01-1995
Permanent home address : Pratap Nagar, Udaipur, Rajasthan

EDUCATIONAL QUALIFICATION

Bachelor's degree : B.Tech. (Dairy Technology)
University : MPUAT, Udaipur, Rajasthan
Year of Award : 2016
OCPA : 7.58/10.00
Master's degree : M. Tech.
OCPA : 8.32/10.00