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Olive leaves extract for functioning dairy products

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Olive leaves extract for functioning dairy products

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Declaration

I certify that this thesis submitted for the degree of master, is the result of my own research, except where otherwise acknowledged, and this thesis has not been submitted for the higher degree to any other university or institution.

Sana Mosbah Hassan Jebreen

Signed: …….

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Abstract:

Green olive leaf was collected from trees type NabaliBaladi localized in several parts in West Bank /Palestine, dried at ambient temperature. Samples were subjected to different extraction method, namely; Simple green (maceration) and new invented extraction green method based on extraction under vacuum and controlled temperature. The highest amount of Oleuropein analyzed by HPLC obtained by two methods was used for further application.

OLE was applied to Cheese, Pudding, and labaneh products in addition of series of different concentrations (0.2%, 0.4% .0.6% and 1%) in base of (w/w). The fortified products were subjected to microbials, sensorial, and shelf-life testing. The type of microbial testing as well as the shelf-life duration was due to the Palestinian standard issued by PSI. The fortified samples were tested for the ability of "OLE" to expand the products' shelf-life and their functionality.

Results showed higher effect for OLE once used in higher addition. Certain dairy products appeared better enhancement in term of texture and consistency once fortified with OLE.The minimum edged of remarkable addition of OLE was the 0.4%.

Using 0.6% and 1% of OLE, Showed completely inhibition of microbial growth at the very early stage until the end of the shelf-life. This pointing result revealed the effect of OLE as success anti-microbial agent for all sample products.

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Abbreviations, Symbols and Terminology:

TPC	Total Plate Count
Y&M	Yeast and mold
F.Coli	Fecal Coliform
Staph	*Staphylococcus aureus*
T.Coli	Total Coliform
Hplc	High-performance liquid chromatography
PFIA	The Palestinian Food Industries Association
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
TBHQ	Tertiary butyl hydroquinone
T.N.T	Too numerous to count
PS	Palestinian Standard
PCA	Plate Count Agar

VRBA	Violet Red Bile Agar
PFIU	Palestinian Food Industries union
OLE	Olive leaf extract

Chapter One

1. General Introduction

Great interest on the functional properties of the natural food or natural ingredients has been focused recently by many researchers(Goldberg, 1996).The studies investigate the therapeutic effects of the biologically active compounds extracted from by- products of natural sources such asplants, food byproductsor even algae and microalgae. That can be used in food preparation and cosmetics applications. A remarkable effect of bioactive compounds has been devoted to the prevention and/or treatment of diseases such as cardiovascular diseases and certain cancer types (Block *et al.*, 1992).However, the protective abilities of bioactive materials are mostly attributed to plant polyphenols and their antioxidant, antimicrobial, antiviral or Anti carcinogenic effects. The concept of functional food was first promoted in 1984 by Japanese scientists who studied the relationships between nutrition, sensory satisfaction, fortification and modulation of physiological systems. In 1991, the Ministry of Health introduced rules for approval of a specific health-related food category called FOSHU (Food for Specified Health Uses) which included the establishment of specific health claims for this type of food. At present, several natural extracts among them that of the olive leaves, is used in the manufacture of functional foods and cosmetics. (Micol*et al.*, 2003).

1.1.Food industry in Palestine

Food industry is considered one of the oldest Industrial sectors inPalestine. Recently, one of the fastest-growing sectors and plays a major role in the Palestinian economy; it contributes more than 24 percent of the production value, 18 percent of the total added value, and 16thfood industry is an essential support for the agricultural sector, serving as a link between Palestinian farmers and domestic and foreign consumers(PFIU).On the otherhand, the Palestinian food-processing plays a very important role in agricultural development since it absorbs the surplus of the agricultural products such as vegetables, fruits, fresh milk, poultry, and olive oilwhich comprise more than 50 percent of the total raw materials used in food processing.

1.1.1. Dairy industry in Palestine :

Dairy processing considered the most important subsector in food processing, before the second Intifada, 60 percent of fresh milk used in Palestinian dairy-processing factories was from Israel. The recent year, all the fresh milk used in dairy plants is from Palestinian cow farms.

Moreover, before the year the 2000.All dairy farms contained fewer than 50 cows; but now days there are more than 10 large farms, each of which has more than 200 milking cows. All these farms use modern farming technology and work in a manner consistent with Good Manufacturing Practice. Fresh milk the Palestinian Food Industries Association encourages dairy farms to maintain good hygienic practices using closed-system milking equipment. (PFIA).

Cheese is one of the most versatile foods suitable for all age groups which can be consumed in many different meal occasions. Traditionally, cheese has been regarded by the consumers as a nutritious food because it is a source of high quality proteins, dietary calcium, fat and other nutrients. The Local white brine cheese is probably the most popular and economically important traditional cheese in Palestine (Messer *et al*., 1985).

1.2. Deterioration of dairy products

One of the most undesirable problems in food industry is the deterioration of the product as a result of Lipid oxidation that lead to the formation of rancid off flavors thus reduce the shelf life of the product . In order to eliminate this problem, the use of synthetic antioxidants, as butylatedhydroxyanisole (BHA), butylatedhydroxytoluene (BHT) and tertiary butylhydroquinone (TBHQ), are widely applicable in the dairy production, in particular cheese products. On the other hand health concerns such as carcinogenic effects of these synthetic antioxidants derive the increasing affinity to the natural sources of antioxidants.

Addition to chemical quality of dairy products, bacterial quality that determine Microbiological safety and shelf life of food materials are strongly correlated.

According to the Palestinian standards, Local White cheese is classified as semi-hard cheese with a moisture content range between (45-55%). In Palestine, this kind of cheese are prepared by boiling cheese pieces, in brine solution (18-20%)before canning and

storage(Yamani et al., 1987). The product then often consumed after several hours soaking in fresh water.

The keeping quality of Local white cheese depends mainly on high salt concentration of storage medium (12.6-26%) and heat treatment by boiling of cheese before storage in tinplate cans (Humeid, 1990). Boiled cheese might be deteriorated during storage due to the development of bad flavor. Deterioration could result from the activity of halophytic bacteria that are able to grow at high salt concentration. To avoided the use of high salt concentration, and increase the keeping quality of the boiled cheese, an increase study was conducted by, (to determine the effect of PH reduction on the shelf life of cheese, found that decreasing the PH of cheese to 4 by the addition of lactic acid permitted the reduction of salt in brine and cheese to 10% concentration, this combination inhibited the growth of bacteria without negatively affecting texture and sensory quality of cheese.

1.3. Olive leaves extract (OLE)

Olive is atypical tree widely cultivated for olive oil production in the Mediterranean area. It considered one of the most important fruit trees in Palestine. numerous studies were carried out on the alimentary use of olive fruits and the olive oil and its important in the daily diet of the human (Pereira *et al.*2007),Moreover, olive leaves and it's important for their secondary metabolites such as the phenolic compounds has been also investigated.(Hansen *et al*., 1996). Phenolic compounds found in olive fruits and leaves vary in qualitative and quantitative terms during the development and ripening process of the fruit (Amiot*et al*., 1989). There is a scientific evidence that olive leaf polyphenols are bioactive compounds and have benefits effects (DeLeonardis*et al*., 2008).Several reports showed that olive leaf extract has a health impact on the human for instance, David Arraez- Rman et al., 2008 found a capacity of OLE to lower blood pressure in vivo coronary arteries many studies researchers demonstrate antiviral (Micol*et al*., 2005), anti-HIV (Lee-Huang*et al*., 2003), antimicrobial (Bisignano*et al*., 1999), antioxidant, anti-inflammatory (Mann et al., 1999; Briante*et al*., 2002), and anti-carcinogenic properties of the OLE. Un addition OLE can stimulatethe thyroid activities and prevent the cancer (Al-Qwarawi*et al*., 2002).

In fact olive leaves became a public health concern due to the large amount of olive leave waste that became a good media for microbial growth and other biological hazardous which became a major concern for public health in the last five years. Never theless, the innovative ideas addressed between Palestinian and the national research institute of Italy, Roma, 2015 focused in recycling the waste of most national health concern, and olive leave extract was the major waste to be recycled and or reused.

1.3.1. The use of the olive leaves extract:

Theuse of olive leave has been made principally through formulations which include crushing of the dried leaves without any previous separation of the active compounds. There are numerous preparations of this type on the market; they are used as complements to the treatment of certain diseases or as nutritional supplements. New patents appear daily for functional foods or cosmetics based on olive leaf extracts. The patents are quite varied in their formulation; for example, there are liquid preparations with alcohol and with glycerin (OlivedaNetwork SL, 2005). Equally diverse are the applications to which these formulations are destined:

- manufacture of dietetic bread for diabetics (Scheneder, 1985).
- Preparations which contain olive leaf extract for use in food or cosmetics (Coll*et al.,* 1999).
- Use of olive leaf extracts in dietetic cookies (Shtukatur,2003).
- The manufacture of a nutritional supplement with physiological effects containing olive leaf extract (Stueckler 1998).
- An extract of olive leaves to combat free radicals, and their use in dietetic foods and in cosmetics (Amari ,1998).

1.4. Objectives of the study

Olive leaves considered as a cheap raw material which can be used as a useful source of high-added value products, this material is a richest source of natural compounds that has a functional properties in term Antioxidant Anti-inflammatory, Anti-atherogenic, Anti-cancer,Anti microbial effects For these reasons, it is commercially available as food supplement in Mediterranean countries. The main objectives of the current study areto:

1- Extract the Palestinian olive leaves obtained from Palestinian olive trees localized in the sunshine area of grassland indifferent region of the west bank .
2- Apply very cheap method of extraction (green extraction)
3- Apply the crude olive leaf extract into dairy products such as:
    a. Pudding
    b. Cheese
    c. Labaneh
    d. And investigate there keeping quality and consumer acceptance
4- Test the effect of olive leave extract on shelf life of dairy products

Chapter Two

2. Literature Review

2.1. Introduction

Inthe last decade, there an increasing interest in researches for production of biologically active compounds from natural sources. Bioactive compounds are remarkable due to prevention and/or treatment of diseases such as cardiovascular diseases and certain cancer types(KeremKaanAytul.2010). The protective abilities of bioactive materials are mostly attributed to plant polyphenols and their antioxidant (Mann et al., 1999), antimicrobial (Bisignano et al., 1999), anti viral(Lee-Huang et al., 2003) or anti carcinogenic effects(Owen et al., 2004). Furthermore, plant polyphenols are preferred as protective ingredients in pharmaceutical, food and cosmetics industries as food additives, preservatives and dietary supplements instead of synthetic chemicals(Medina et al., 2006; Nagayamaet al., 2002).

2.2.*Oleaeuropaea L*

The medicinal properties of the olive tree were discovered from the early 1800s, when it was used in liquid form for malaria treatment (Wern*et al.,* 1985). In 1900s a bitter compound "oleuropein," was determined in the leaves of certain olive trees andwas thought to have certain medical benefits (Walker *et al*., 1997).

2.2.1. Olive Leaves Extract:

Olive leaf extract was foundto contain many phytochemicalsthat have might improve the resistant properties of olive treeto insects and bacterial damage (Pooley*et al*., 1997).In 2000 a research performed by Somova*et al*., determine the importance of the *Oleaeuropaea*L. Leaves as a typical herbal drug used widelyin the Mediterranean are as traditional medicine forvasodilator, hypotensive, anti-inflammatory, anti-rheumatic, diuretic, antipyretic, and hypoglycemic agents. Benavat*et al*., 2000, highlighted the wide number of active constituents of the olive leaf including chief the constituent “Oleuropein (60-90 mg/g). And several types of polyphenolic compounds. (Table 2:1).

The following polyphenols have been also detected in olive leaf tissue: hydroxytyrosol, tyrosolelenolicacid derivatives, caffeic acid, oleuropein, verbascoside,luteolin, apigenin-7-O-rutinoside and apigenin 7-O-glucoside,There are atleast six active substances (Oleuropein, hydroxytyrosol, caffeic acid, vanillin, luteolin-7-glucoside, and verbascoside) also found in the extract. These substances work together synergistically to decrease pathogen microorganismsresistanceto antibiotics. However, Oleuropein was most studied component among polyphenols, in fact the amount of Oleuropein in olive leaves affected by several factors, including Oleaeuropea variety, time of collection, possible infestation by olive fly "DacusOlea", climate, storage,conditionsand the method of extraction. Oleuropein content varies from17% to 23% depending upon the time of year the leaves are harvested (Le toutour*et al.*, 1992).

Table 2:1Phenolic compounds found in OLE, and their relative amounts:

Group Name	Example Compound	% Amount in OLE
Oleuropeosides	Oleuropein	24.54
	Verbascoside	1.11
Flavones	Luteolin-7-glucoside	1.38
	Apigenin-7-glucoside	1.37
	Diosmetin-7-glucoside	0.54
	Luteolin	0.21
	Diosmetin	0.05
Flavonols	Rutin	0.05
Flavan-3-ols	Catechin	0.04
Substitued Phenols	Tyrosol	0.71
	Hydroxytyrosol	1.46
	Vanilin	0.05
	Vannilic acid	0.63
	Caffeic acid	0.34

- **Terpenic compounds in olive leaves:**

In 1969, determined the presence of terpenic acids in the Olive Leafina considerable amount accounting for 3% of dry leaf weight (Albi*et al*., 2001)

The Oleanolic acid was mentioned in a research study by (Saady*et al*., 1994).to have abiologically active anti-abortive, anti-cariogenic, anti-fertility,antihepatotoxic, anti-inflammatory, cancer prevention, cardiotonic, diuretic, hepatoprotective properties

Liposoluble compounds in olive leave:

In 1973, a liposoluble compounds were isolated from the olive leaves by thin layer chromatography using hexan extraction. The main liposoluble compounds present in olive leaves are:

Saturated hydrocarbons, Squalene, ester Ester waxes, Alpha-tocopherol, Triglyceride, Beta carotene, linear alcohols, Alpha-and beta- amyrine, and Beta-sytosterol.

These compounds have multiple applications in the pharmaceutical, cosmetics, and food additives industrials.

Other Compoundsfound in the olive leaves contain Mannitol(Wisselink*et al*., 2002). Ahexitol derivatives of mannose. With advantageous applications in the food and pharmaceutical industries. It's sweetening potency equivalent to 70% of sucrose sweetness; it has a low caloric value (2kcal/g), which makes it suitable for consumption by diabetics. Additionally, it has healthful effects as an antioxidant and is of therapeutic use in severe head injuries, as it is effective in the reduction of intracranial pressure (Unterberg *et al*., 1997).

2.2.2. Functional proprieties of OLE:

Beneficial properties of olive leaf extracts are further enhanced by the bioavailability of their polyphenolic constituents, and their high absorptivity through the gastrointestinal tract(Visioli and Galli, 2000; Visser*et al*., 2002). Regarding human health, much concern has been focused on phenolic compounds from plants source that may modulate microbiota in the intestine by selectively increasing that growth of *bifid bacteria*and *lactobacilli* and decreasing and preventionthe growth ofharmful bacteria such as clostridia.

2.2.2.1.Antioxidant activity of polyphenols:

Because of their content of polyphenols, olive leave extracts shown to have an antioxidant capacity of 400% higher than vitamin C and almost double that of green tea or grape seed extract (Ryan and Robards, 1998).

Oleuropein has high antioxidant activity in vitro, in comparison withtocopherol. Furthermore,oleuropein scavenges superoxide anions and hydroxyl radicals, and inhibits the respiratory burst of neutrophils and hydrochlorous acid-derived radicals (Visioli*et al*., 2002).

2.2.2.2. Antimicrobial Properties of polyphenols:

In addition to its antioxidant properties, phenolic compounds have shown to have antimicrobial activities against several microorganisms including;E. *coli*, *Staphylococcus aureus*, *Klebsiellapneumoniae*, *Bacillus cereus*, *Salmonellatyphi*and*Vibrio parahaemolyticu*(Markin et al., 2003). In addition, OLE affectsmacrophage function and modulates inflammatory response; those may contribute toactivity against infectious (Lee-Huang et al., 2003).Although the individual phenolic compounds in olive leaf extract may demonstratestrong activities in vitro, the antioxidant and antimicrobial activities of combinedphenolics showed similar or better effects than the individual phenolics (Lee at al.2010).

Pereira et al., (2007) indicated that, OLE extractshavemore beneficial properties to human health than isolated constituents, since a bioactive component can change itproperties in the presence of other compounds present in the extract.Moreover, they reportedthe antimicrobial capacity for several concentrations of OLE as followagainst different kind of bacteria in the following order; *B. cereus~C. albicans> E. coli> S. aureus> C. neoformans~ K. pneumoniae~ P. aeruginosa>B.subtilis.*

Markin et al., (2003) also indicated that, water extract of olive leaf with aconcentration of 0.6% (w/v) prevent*Escherichia coli*, *Ps. aeruginosa*, *S. Aureus*and*K. Pneumonia*in after 3h exposure. *B. Subtilis*onthe other hand can be inhibited only when the concentrationwas increased to 20% (w/v) possibly due to spore forming ability of this species.

Sudjana et al., (2009), studied the antibacterial activity of olive leaf extract withlarge variety of bacteria, the results showed that OLE did not present wide-spectrumantibacterial activity, but had appreciable activity on *H. pylori* and *C. jejuni*.

2.3. Application of OLE in food

Ali Ahmed *et al.,*2014. study, the effect of olive leaves extract (OLE) on the microbial load of raw peeled undeveined (PUD) shrimp (Penaeussemisulcatus) and found that the usage of 2% OLE had the most beneficial effect in controlling microbial load in PUD shrimp stored at 4 ºC.

Another study in 2010 by KeremKaanAytul. showed that Appling OLE to raw beef cubes with 1%, 2% and 3% (v/w) concentrations to examine its antimicrobial and antioxidant effects. The results clearly indicated that usage of 2% and 3% OLE had the beneficial effect in controlling the microbial load, total viable and coliform counts, of beef cubes during 9 days of storage at 4ºC. And in the same study, 300 ppm OLE was applied to sardine (*Sardinapilchardus*) fillets as a marinade component. Results indicated that OLE was effectivein controlling microbial load of sardine fillets and also delayed the oxidativedeterioration.

Mohammad Marhamatizadeh*et al*in 2013. Investigated the effect of olive leaf extract on Growth and viability of *Lactobacillus acidophilus* and *Bifidobacteriumbifidum*in milk and yoghurt during 21 days refrigerated storage. In order to determine the effect of different doses of olive leaf extract on growth and viability of probiotic bacteria in milk and yoghurt. With different concentration of OLE (0.2, 0.4 and 0.6%). The results showed that the samples containing *Lactobacillus acidophilus* and *Bifidobacteriumbifidum*, it was observed that increased concentrations of olive leaf extract create a favorable taste in milk and yoghurt. And the samples with 0.6% olive leaf in milk and yoghurt had greater viscosity than the other samples investigated

Chapter Three

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3. Materialsand Methods

3.1 Introduction:

This chapter will discuss all materials used in the approved methods enable to perform the preset research methodology which express the mentality of the research.

The materials used in this investigation included; materials used for sample preparation, materials used for analysis, and materials used for extraction.

While methods divided into olive leaves preparation method, sample processing methods, sensorial evaluation methods, analytical methods, and extraction method.

It is very important to show the harmony between the materials and the methods applied. However, each method applied is a major part to conduct the methodology.

3.2 Materials:

3.2.1 Materials used for sample processing:

The materials used are limited for producing cheese, pudding, and labaneh. The materials are listed in Table 3:1.

Table 3:1 Materials used in sample processing methods

No.	Material type	Characteristics	Source
1	Cow milk	Fresh non pasteurized	Local farm
2	Starter culture	Mother culture	Local shop
3	Rennet enzyme	Liquid form	ADS factory
4	$CaCl_2$	Coarse particle	Sun Company for food raw materials Nablus, Palestine

5	Starch,	Standard	Sun Company for food raw materials (Palestine)
6	Gelatin	Fish gelatin	Sun Company for food raw materials (Palestine)
7	Carragenan	Standard	Sun Company for food raw materials (Palestine)
8	NaCl	Standard particle size, pure salt	Local shop

3.2.2 Material used for analysis:

The materials used are limited for microbial analysis, HPLC chromatographic analysis, as listed in Table 3:2.

Table 3:2 Materials used in analytical methods

No.	Material type	Characteristics	Source
1	Hydrochloric acid	65% concentration	Biotech Company
2	Distilled water	Chromatographic grade-double,	Alquds university
3	Acetonitrile	HPLC grade	Merck
4	acetic acid	analytical grade	Sigma and Aldrich
5	Sodium hydroxide	analytical grade	Sigma and Aldrich
6	Hydrochloric acid	analytical grade	Sigma and Aldrich
7	citric acid	analytical grade	Sigma and Aldrich
8	calcium chloride	analytical grade	Sigma and Aldrich
9	ethanol	analytical grade	Sigma and Aldrich

3.3 Methods:

3.3.1 Method used for olive leave extraction:

Olive leave extraction method passed in several steps until the olive leave extract was obtained. These steps will be discussed separately.

3.3.1.1 Olive leave collection:

Olive leaves samples were obtained from trees type NabaliBaladi localized in several parts in West Bank /Palestine. The collection was directly from the trees from an old branch exactly closer to the stem in November, December, January and February 2015-2016.

3.3.1.2 Olive leave preparation:

Fresh leaves were dried at ambient temperature. Then the dried samples were grinded to obtain powder which was stored at room temperature in dark until extraction.

3.3.1.3 Extraction method:

The green method of extraction, resulted from previous research performed by the research team, will be considered for preparing the OLE directed for food implementation.

4kgm of olive leaves powder were macerated in 40liter solvent for 4 hours. The solvent mixtures used for the extraction were: Deionized water at pH (3). (Adjusted with Hydrochloric acid (0.1N), at 40°C. The extracts were then filtered to separate coarse particles from the solutions. The filtered extracts were then evaporated at 40°C in open atmosphere (old method), but under vacuum in double jacket tank (new method). The concentrated extracts were stored in a refrigerator at (2-4°C) until used, as shown in Figure 3:1.

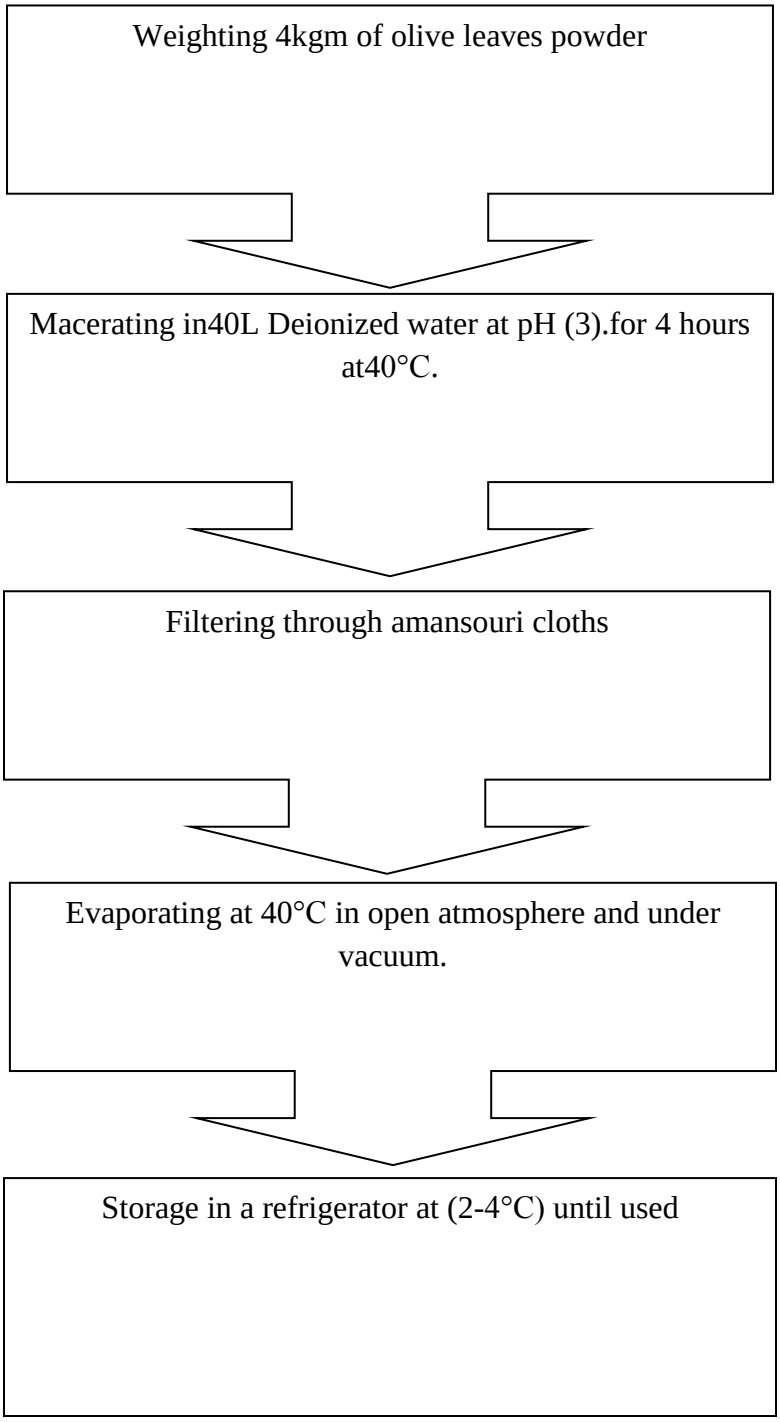

Figure 3:1 Flow chart for olive leave extraction method

3.3.2 Methods used for sample processing:

As this research investigated dairy product, thus three major different dairy products were determined to be investigated in this research. Method of sample processing included; cheese, pudding, and labaneh.

3.3.2.1 Method used for producing local white cheese sample:

Local white cheese was manufactured using fresh raw milk which was obtained from a dairy farm, Palestine. Milk was pasteurized at 75°C for 15 second using a double jachet container and cooled at (39°C - 41°C). Then milk was transported carefully to a cheese vat with the temperature of 38°C.

OLE with different concentrations (0.0%, 0.2%, 0.4%, 0.6%) and 1%, were added to the milk, then milk was supplemented with 0.15 g of $CaCl_2$/kg of milk and held at 35°C until the final pH of milk reached 6, 6.2 and 6.4 before the addition of rennet. The illustrated process is shown in Figure 3:2.

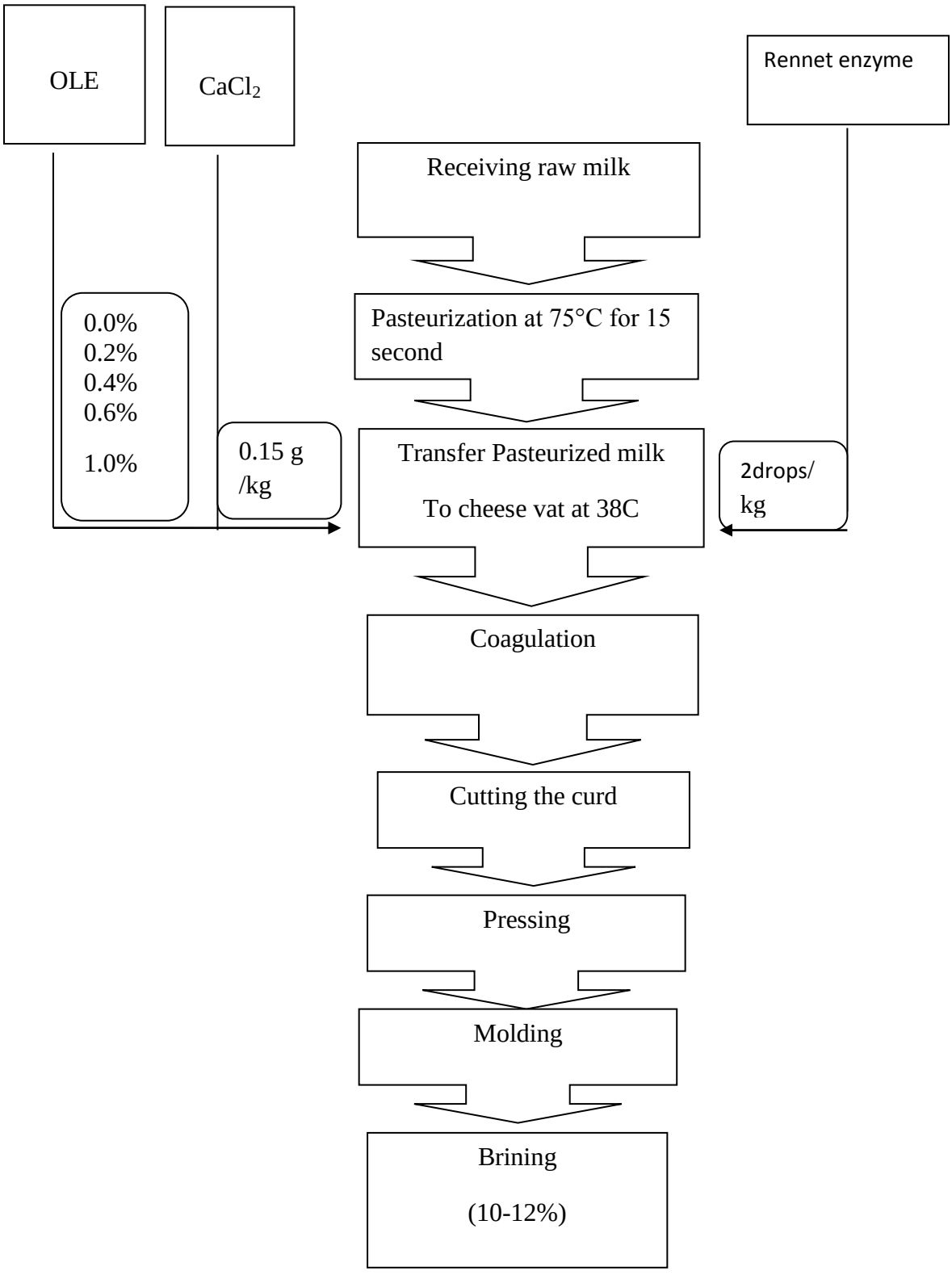

Figure 3:2 Flowchart applied to produce local white cheese

3.3.2.2 Method used for producing Pudding sample:

Pudding was manufactured using fresh raw milk which was obtained from a dairy farm, Palestine. Milk was pasteurized at 75°C for 15 second using a double jacket container and cooled at (39°C - 41°C).

Then OLE with different concentrations (0.0%, 0.2%, 0.4%, 0.6%) and1%, were added to the milk, then milk was supplemented with 0.6% Starch, 1.5%Carragenan and. Sugar and chocolate were add to enhance the color and flavor (shown in Figure 3:3).

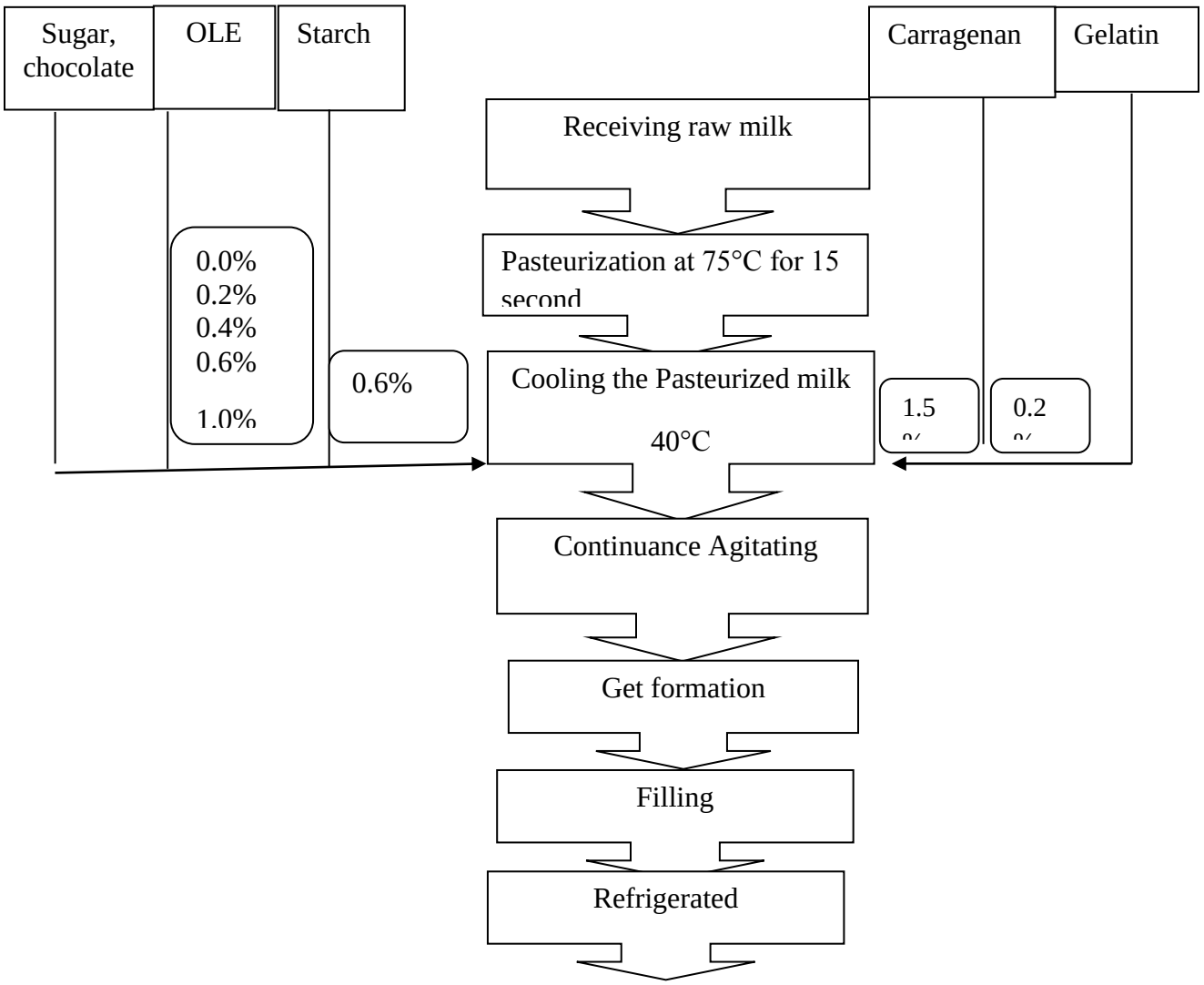

Figure 3:3 flow chart for pudding processing

3.3.2.3Method used for producing Labaneh sample:

Labaneh was manufactured using fresh raw milk which was obtained from a dairy farm. Milk was pasteurized at 95°C for 15 second using a double jacket container and cooled at (40-42°C). OLE with different concentrations (0.0%, 0.2%, 0.4%, 0.6%) and 1%, were added to the milk, then milk was inculated with the mother culture at 42°C and held for 4 hours. The illustrated process is shown in Figure 3:4.

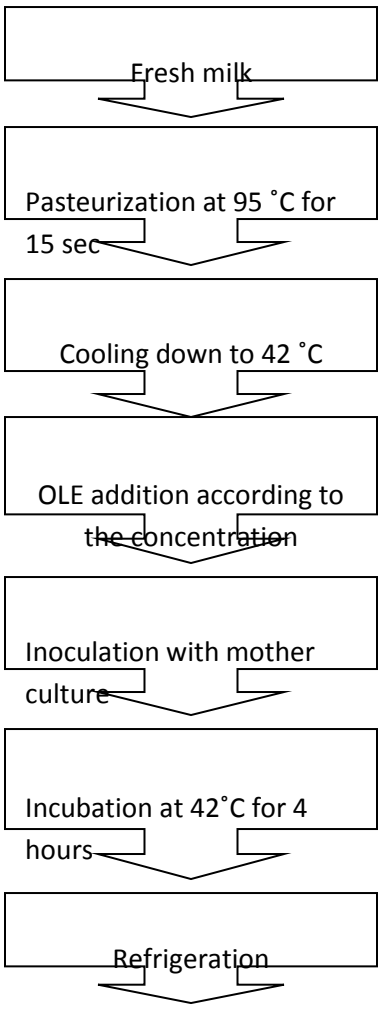

Figure 3:4 flowchart for labaneh processing

3.3.3 Sensorial evaluation Method:

Samples were sensorial evaluated during storage for 0, 2, 4, 6, and 8 weeks according to standard method of Panel testing (Papas *et al*., 1996). The evaluation was carried out by score from 1 to 9 were 9 is the best scoring. The samples were subjected for several criteria evaluation including, taste, texture, consistency, mouth feeling, ,…etc. Two levels of evaluation were carried out, accordingly;

- At Research level: were the total samples produced tested and evaluated by two researcher.
- At Market level: random sample of 100 adults customers were used to carry out this evaluation. Samples were tasted and physically tested. Customers were asked to taste and destructive checking up the samples enable to rank the addressed samples in order of preference. The addressed samples were introduced under coding.

The note and preference of the panelist listed in the questionnaire were analyzed. The provided questionnaire is shown in Table 3:3.

Table 3.3 questionnaire provided for sensorial evaluation

No.	Sample code	General appearance	Texture	Consistency	Taste	Color	Moth feeling	After taste	Gross acceptance

3.3.4 Methods used for analysis:

3.3.4.1Shelf-life product Analysis:

3.3.4.1.1 Microbial analysis:

The measurements published by the PSI. Microbial tests were. A standard product without additives and fortification will be used as a reference. Cheese, Labaneh and pudding were sampled for analysis at the age of 0, 1, 2, 3, 4, 5, 6, 7, 8 weeks.

The total viable microflora in cheese and pudding was enumerated by the pour-plate method using plate count agar, violet red bile agar. Malt extract agar and baird parker agar. (Merck, Darmstadt, Germany). The plates were incubated respectively at 35 _C ,44C,25 C, 35.(IDF 100B:1991) and microbiological count data was expressed as log10 of colony-forming units (cfu) per ml or g. The enumeration was performed on milk or yogurt just after the inoculation with oleuropein, and then after 4 and 7 days for milk and after 14 and 35 days for yogurt at 4 ˚C. All determinations were made induplicate.

3.3.4.1.2 Physical Appearance:

All stored samples were checked up for any physical disorder or up normal appearance. The following features were tested:

- Package swelling
- Molding characteristics
- Change in color
- Change in consistence
- Water synergism
- Off odor

3.3.4.2 Olive Leaf Extract Analysis:

One of the purposes of this investigation was to evaluate the invention of new method of extraction for olive leave extract. OLE obtained by the new invented method were compared with OLE obtained from the traditional extraction method.

The HPLC analysis was used for the determination of phenolic compounds andespecially for the quantification of oleuropein in two extracted samples. The HPLC equipment used was aHewlett-Packard Series HP 1100 equipped with a diode array detector.

The stationaryphase was a C18 LiChrospher 100 analytical column (250 mm×4 mm i.d.) with aParticle size of 5 mm thermostated at 30 ºC. The flow rate was 1 mL/min and theAbsorbance changes were monitored at 280 nm.Oleuropein in OLE was identified by comparing its retention times with the corresponding standards.

3.4.Methodology

Olive leaves extracted as a plant source of functional materials. A critical step for fortification was due to the effectiveness of OLE (i.e the concentration of Oleuropein). New invented method for extraction was applied based on the ability of extraction to extract highest amount of Oleuropein. This new extraction method applied by using a double jacketed container with controlled temperature and vacumized atmosphere applied under relatively gentle vacuum to remove air for the head space of the agitated maceratedmatrix, as shown in Figure 3:5

Figure 3:5 The new invented apparatus for olive leave extraction.

The obtained OLE from this new invented method was compared with OLE obtained from traditional method of extraction.

The huge Oleuropein content of the OLE obtained by new invented method in comparison with its content in OLE obtained by traditional method was the driving force behind using it and excluding the use of OLE obtained by traditional method.

Applied the crude olive leaves extract in different concentrations to compare the added value of the fortification step in correlation with sensorial analysis into different dairy products:

a) White cheese
b) Pudding
c) labaneh

Then measurements and analysis for shelf-life of products will be according to testing the standard shelf-life published by the PSI. Microbial and sensorial tests will be conducted. A standard product without additives and fortification will be used as a reference.

The fortified samples' shelf-life investigation as well as with the sensorial evaluation were determined the effectiveness and the impact of using OLE as a fortification for the studied dairy products.An overview of the experimental investigations is presented in Figure 3:6

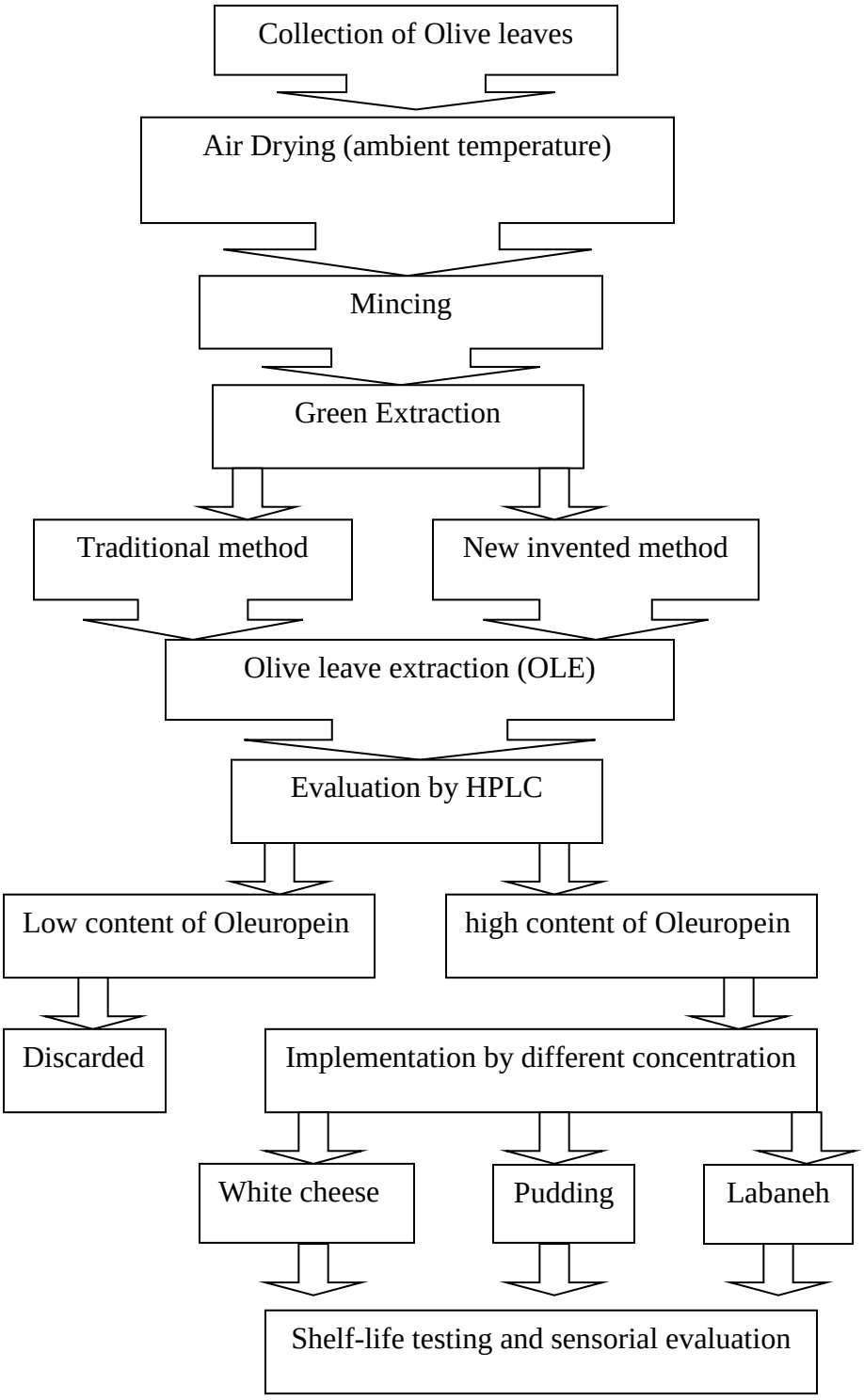

Figure 3:6 schematic diagram for the methodology applied in investigating the effectiveness of OLE in prolonging dairy product shelf-life.

Chapter Four

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4. Results and discussion

4.1 Introduction

This part of investigation will discuss the result obtained in the life of the research. The results obtained will be described and thus evaluated and weighted by comparing it with the citied results in the literature.

The results approved to be discussed in this chapter were limited to results obtained for three categories, namely; cheese, budding,Labaneh and extraction method comparison between old and new invented method.

However, for cheese , Labanehand budding categories each of which will be discussed in term of microbial stability, sensorial evaluation, and quality of shelf life.

4.2. Investigation in Cheese

4.2.1 The Antimicrobial effect of OLE on Cheese Product:

Table 4:1 Microbial growth for cheese sample stored at 5 ˚C without OLE fortification, expressed as CFU

Time	TPC	Y&M	STAPH	F.COLI	T.COLI
0Week	Nil	Nil	Nil	Nil	Nil
1Week	Nil	100	Nil	Nil	10
2Weeks	100	100	50	Nil	30
3Weeks	125	200	80	Nil	50
4Week	T.N.T.C	T.N.T.C	110	Nil	150
8Weeks	T.N.T.C	T.N.T.C	150	Nil	200

Result for standard cheese sample (without fortification) were plotted in Table 4:1 showed that, cheese sample showed an ordinary trend of microbial growth during storage for eight weeks. As expected the sample showed high microbial growth in form of TPC and fungus after the expiry date. The coliform growth was highly remarked as well.

This term of microbial development comes in accordance with the normal manifested microbial growth for such product.

Same finding were reported by *Melilli et al*. (2004) who worked on fatta cheeses and found that; low initial salt and higher brining temperature (18 ˚C) allowed for greater growth of coliforms.

Table 4:2 Microbial growth for cheese sample stored at 5 ˚C fortified with 0.2% (w/w) OLE, expressed as CFU

Time	TPC	Y&M	STAPH	F.COLI	T.COLI
0Week	Nil	Nil	Nil	Nil	Nil
1Week	Nil	Nil	Nil	Nil	Nil
2Weeks	100	100	20	Nil	30
3Weeks	140	120	60	Nil	50
4Week	160	170	80	Nil	70
8Weeks	T.N.T.C	T.N.T.C	100	Nil	120

Table 4:2 showed that the microbial growth started after two weeks in form Yeast and mold and Total coliform. While by the time of 8 weeks storage showedhigh number of Yeast and mold growth and relatively high number of Total Plate Count. At the same time the addition of 0.2% OLE appear to have limited effect on microbial prevention growth. While the of 0.4% OLE delayed the same microbs until weekfour

This inhibition is due to the addition of OLE, this result compatible with Pereira et al., (2007) who revealed that the growth rates of *S. aureus*were decreased while OLE concentration increased.

Table 4:3 Microbial growth for cheese sample stored at 5 ˚C fortified with 0.4% (w/w) OLE, expressed as CFU

Time	TPC	Y&M	STAPH	F.COLI	T.COLI
0Week	Nil	Nil	Nil	Nil	Nil
1Week	Nil	Nil	Nil	Nil	Nil
2Weeks	Nil	Nil	Nil	Nil	Nil
3Weeks	Nil	Nil	Nil	Nil	Nil

4Week	50	40	Nil	Nil	30
8Weeks	80	60	20	Nil	50

Table 4:4 Microbial growth for cheese sample stored at 5 ˚C fortified with 0.6% (w/w) OLE, expressed as CFU

Time	TPC	Y&M	STAPH	F.COLI	T.COLI
0Week	Nil	Nil	Nil	Nil	Nil
1Week	Nil	Nil	Nil	Nil	Nil
2Weeks	Nil	Nil	Nil	Nil	Nil
3Weeks	Nil	Nil	Nil	Nil	Nil
4Week	Nil	Nil	Nil	Nil	Nil
8Weeks	Nil	Nil	Nil	Nil	Nil

Result of cheese samples fortified with 0.6% (w/w) OLE showed in Table 4:4. The results obtained expressed a high effect of OLE on microbial growth. At the very early stage of storage, samples showed a high stability on the growth of microbial status. This stability was continued until the end of the shelf-life time.

This remarkable result revealed the hypothesis of the role played by OLE as success anti-microbial agent. Even more, the ordinary growth of Staph and Y&M appeared in the non-fortified sample were completely inhibited by the addition of 0.6% OLE, for the predetermined shelf-life of the sample. Which compatiblewithMarkin et al., (2003)whoreported that water extract of olive leaf with a concentration of 0.6% (w/v) killed E.coli, P. aeruginosa, S. aureus and K. pneumonia in 3h exposure.

Table 4:5 Microbial growth for cheese sample stored at 5 ˚C fortified with 1% (w/w) OLE, expressed as CFU

Time	TPC	Y&M	STAPH	F.COLI	T.COLI
0Week	Nil	Nil	Nil	Nil	Nil
1Week	Nil	Nil	Nil	Nil	Nil
2Weeks	Nil	Nil	Nil	Nil	Nil

3Weeks	Nil	Nil	Nil	Nil	Nil
4Week	Nil	Nil	Nil	Nil	Nil
8Weeks	Nil	Nil	Nil	Nil	Nil

The above results presented the using of 1% of OLE. It showed that the same effects on microbial growth as appeared in 0.6% addition, which confirmed the effect of OLE as a successful antimicrobial agent. This result comes in accordance with Ali M. Ahmed et al,2014.Who reported a high antimicrobial effect for OLE on preserving shrimp (Penaeussemisulcatus) products agents microbial growth when he used a high concentration of OLE reached up to 2%. This high concentration was due to a high protein and aqueous matrix (fish), while stronger and more stable protein as, in case of cheese (high impact matrix), much lower concentration were effective.

4.2.2. The Sensorial evaluationof Cheese Products:

The sensory evaluation of cheese samples after fortification with OLE extract (table 4:6) showed that the addition of OLE negatively affect the taste, flavor, and color of the cheese samples, in this regard, the evaluation records were decreased dramatically as the OLE fortification percent increased. These results could be explained by the bitter taste of theoleuropein in the OLE,Syed H. (2010), and the dark color of the added extract. The sensory results of the texture were observed to be positively affected (Table 4:6) as the OLE added percent increased, were the texture has been described by the participants as "favored elasticity".

Table 4:6 Sensorial evaluation of cheese samples fortified with different concentrations of OLE

	Ranking (% of participant)								
Characteristics	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Standard sample without fortification									
Taste	0	0	0	0	0	0	0	20	80
Flavor	0	0	0	0	0	0	10	10	80
Color	0	0	0	0	0	0	10	0	90
Texture	0	0	0	0	0	30	0	10	60
Cheese sample fortified with 0.2% OLE									
Taste	0	0	60	10	20	10	0	0	0
Flavor	0	0	0	10	50	40	0	0	0
Color	0	60	10	20	10	0	0	0	0
Texture	0	0	0	40	30	30	0	0	0

Cheese sample fortified with 0.4% OLE									
Taste	0	60	20	20	0	0	0	0	0
Flavor	0	40	30	10	20	0	0	0	0
Color	50	30	20	0	0	0	0	0	0
Texture	0	0	0	50	20	30	0	0	0
Cheese sample fortified with 0.6% OLE									
Taste	70	20	10	0	0	0	0	0	0
Flavor	0	70	30	0	0	0	0	0	0
Color	90	10	0	0	0	0	0	0	0
Texture	0	0	0	0	0	80	20	0	0
Cheese sample fortified with 1% OLE									
Taste	90	10	0	0	0	0	0	0	0
Flavor	90	10	0	0	0	0	0	0	0
Color	100	0	0	0	0	0	0	0	0
Texture	0	0	0	0	0	0	90	10	0

4.2.3. The shelf life of Cheese Products fortified with OLE:

Shelf-life testing is essential in determining the quality of a product over time, how long that product is fit for consumption, and indicates if reformulation is required.

According to the microbial results table 4:4 and the observation of the samples after storage at 5 ^{o}C for 2 months, it might be highlighted that the products (cheese) shelf life and keeping quality was improved with the addition of OLE due to the effect of phenolic compounds that retard the oxidation, Visioli et al., (2002).And the OLE antimicrobial activity.Owen et al. (2000).

4.3. Investigation of Pudding

4.3.1. The antimicrobial effect of OLE on Pudding product:

Table 4:7 Microbial growth for pudding sample stored at 5 ˚C without OLE fortification, expressed as CFU.

Time	TPC	Y&M	STAPH	F.COLI	T.COLI
0Week	Nil	Nil	Nil	Nil	Nil
1Week	Nil	Nil	Nil	Nil	Nil
2Weeks	10	Nil	Nil	Nil	Nil
3Weeks	50	10	Nil	Nil	Nil

4 Weeks	80	50	Nil	Nil	10
8 Weeks	150	200	Nil	Nil	30

The above Table showed the result for standard pudding sample (without fortification)which expressedthat the microbial growth started from the early stage of storage in form Yeast and moldand Total plate count. While the other microbes were under the acceptable number during the storage time.

Table 4:8 Microbial growth for pudding sample stored at 5 ˚C fortified with 0.2% (w/w)OLE, expressed as CFU

Time	TPC	Y&M	STAPH	F.COLI	T.COLI
0Week	Nil	Nil	Nil	Nil	Nil
1Week	Nil	Nil	Nil	Nil	Nil
2Weeks	Nil	Nil	Nil	Nil	Nil
3Weeks	20	Nil	Nil	Nil	Nil
4Week	40	Nil	Nil	Nil	Nil
8Weeks	90	100	Nil	Nil	Nil

The results above showed that the microbial growth of yeast and molddelayedto appear until week eight. At the same time addition of 0.2% OLE succeed to inhibit the other tested microbesduring the storage time.

Table 4:9 Microbial growth for pudding sample stored at 5 ˚C fortified with 0.4% (w/w)OLE, expressed as CFU

Time	TPC	Y&M	STAPH	F.COLI	T.COLI
0Week	Nil	Nil	Nil	Nil	Nil
1Week	Nil	Nil	Nil	Nil	Nil
2Weeks	Nil	Nil	Nil	Nil	Nil
3Weeks	Nil	Nil	Nil	Nil	Nil
4Week	Nil	Nil	Nil	Nil	Nil
8Weeks	Nil	Nil	Nil	Nil	Nil

Table 4:9 showed that the results obtained expressed completely inhibition of microbial growth at the very early stage until the end of the shelf-life by the addition of 0.4% (w/w) OLE. This pointing out the conformity of the role played by OLE as successfulanti-microbial agent.

Table 4:10 Microbial growth for pudding sample stored at 5 ˚C fortified with 0.6% (w/w) OLE, expressed as CFU

Time	TPC	Y&M	STAPH	F.COLI	T.COLI
0Week	Nil	Nil	Nil	Nil	Nil
1Week	Nil	Nil	Nil	Nil	Nil
2Weeks	Nil	Nil	Nil	Nil	Nil
3Weeks	Nil	Nil	Nil	Nil	Nil
4Weeks	Nil	Nil	Nil	Nil	Nil
8weeks	Nil	Nil	Nil	Nil	Nil

Table 4:11 Microbial growth for pudding sample stored at 5 ˚C fortified with 1% (w/w) OLE, expressed as CFU

Time	TPC	Y&M	STAPH	F.COLI	T.COLI
0Week	Nil	Nil	Nil	Nil	Nil
1Week	Nil	Nil	Nil	Nil	Nil
2Weeks	Nil	Nil	Nil	Nil	Nil
3Weeks	Nil	Nil	Nil	Nil	Nil
4Weeks	Nil	Nil	Nil	Nil	Nil
8weeks	Nil	Nil	Nil	Nil	Nil

The above results as shown in tables, 4:9, and 4:10) presented that using 0.6% and 1% of OLE, Showed that the results obtained expressed completely inhibition of microbial growth at the very early stage until the end of the shelf-life. This pointing result revealed the effect of OLE as success anti-microbial agent.

4.3.2. The Sensorial evaluationof Pudding Products:

Table 4:12 Sensorial evaluation of pudding samples fortified with different concentrations of OLE

	Ranking (% of participant)								
Characteristics	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Standard sample without fortification									
Taste	0	0	0	30	30	40	0	0	0
Flavor	0	0	0	0	50	40	10	0	0
Color	0	0	0	0	40	10	30	20	0
Texture	0	0	0	0	0	30	60	20	0
Pudding sample fortified with 0.2% OLE									
Taste	0	0	60	10	20	10	0	0	0
Flavor	0	0	0	10	50	40	0	0	0
Color	0	0	60	20	10	10	0	10	0
Texture	0	0	0	0	30	30	0	40	0
Pudding sample fortified with 0.4% OLE									
Taste	0	0	0	0	20	60	10	10	0
Flavor	0	0	0	0	0	50	40	10	0
Color	0	0	0	0	0	0	40	20	50
Texture	0	0	0	0	0	0	10	20	70
Pudding sample fortified with 0.6% OLE									
Taste	0	0	0	0	20	60	10	10	0
Flavor	0	0	0	0	0	50	40	10	0
Color	0	0	0	0	0	0	40	20	50
Texture	0	0	0	0	0	0	10	20	70
Pudding sample fortified with 1% OLE									
Taste	0	0	0	0	20	60	10	10	0
Flavor	0	0	0	0	0	50	40	10	0
Color	0	0	0	0	0	0	40	20	50
Texture	0	0	0	0	0	0	10	20	70

The table above showed the effect of OLE additional for the sensorial evaluation of pudding product, the results appeared undesirable change in colorstarts from 0.2%, while the texture enhancing at the same concentration. As OLE concentrationincrease the taste and color be more acceptable because the addition of flavoring agents (chocolate).

4.3.3. The shelf life of pudding Products fortified with OLE:

According to the microbial results table 4:9 and the observation of the samples after storage at 5 oC for 2 months, it might be highlighted that the products (pudding) shelf life and keeping quality was improved with the addition of OLE due to the effect of phenolic compounds that retard the oxidation, Visioli et al., (2002). And the OLE antimicrobial activity. Owen et al. (2000).

4.4. Investigation in Labaneh

4.4.1 The Antimicrobial effect of OLE on Labaneh Product:

The standard labaneh samples prepared in laboratory without OLE fortification were subjected for microbial test.The samples showed an ordinary trend of microbial growth during the storage time. The results obtained, as shown in Table 4.13 revealed that the sample started to demonstrate microbial growth after four months in form of yeast and mold. This growth allowed the initiation of Staph and T.Coli to regenerate which started after one month of storage.

Table 4:13 Microbial growth for Labaneh sample stored at 5 ˚C without OLE fortification, expressed as CFU

Time	Y&M	STAPH	F.COLI	T.COLI
0Week	Nil	Nil	Nil	Nil
1Week	Nil	Nil	Nil	Nil
2Weeks	10	Nil	Nil	Nil
3Weeks	50	Nil	Nil	Nil
4Week	200	10	Nil	10
8Weeks	T.N.T.C	120	Nil	80

The fortified labaneh sample with different concentration of OLE were investigated by fortifying with 0.2%, 0.4%, 0.6%, and 1%. The results plotted in Table 4:14, Table 4:15, Table 4:16, and Table 4:17, respectively.

Table 4:14 Microbial growth for Labaneh sample stored at 5 ˚C fortified with 0.2% (w/w) OLE, expressed as CFU

Time	Y&M	STAPH	F.COLI	T.COLI
0Week	Nil	Nil	Nil	Nil
1Week	Nil	Nil	Nil	Nil
2Weeks	Nil	Nil	Nil	Nil
3Weeks	10	Nil	Nil	Nil
4Week	50	Nil	Nil	10
8Weeks	200	Nil	Nil	30

Studying Table 4:14 shows that the addition of 0.2% OLE had no effect on retarding or inhibiting the microbial growth in Labaneh.

At the same time the higher addition of OLE (i.e. 0.4%) expressed a certain tolerance for microbial growth as shown in Table 4:15. This limited inhibition for microbial growth granted due to the effect of total phenols in OLE as good antimicrobial agent.

Table 4:15 Microbial growth for Labaneh sample stored at 5 ˚C fortified with 0.4% (w/w) OLE, expressed as CFU

Time	Y&M	STAPH	F.COLI	T.COLI
0Week	Nil	Nil	Nil	Nil
1Week	Nil	Nil	Nil	Nil
2Weeks	Nil	Nil	Nil	Nil
3Weeks	Nil	Nil	Nil	Nil
4Week	60	Nil	Nil	10
8Weeks	180	10	Nil	20

The higher amount of OLE (0.6% and 1%) showed higher effect in retarding the growth of microbs presents in Labaneh. It is obvious from results obtained for both fortification concentration, the high concentration (0.6%) andthe elevated concentration (1%), the effect of OLE through oleuropein was very high in prohibiting the microbial growth in all studied form. These results reveal the advantages of fortifying labaneh sample with such concentration.

Table 4:16 Microbial growth for labaneh sample stored at 5 ˚C fortified with 0.6% (w/w) OLE, expressed as CFU

Time	Y&M	STAPH	F.COLI	T.COLI
0Week	Nil	Nil	Nil	Nil
1Week	Nil	Nil	Nil	Nil
2Weeks	Nil	Nil	Nil	Nil
3Weeks	Nil	Nil	Nil	Nil
4Week	Nil	Nil	Nil	Nil
8Weeks	Nil	Nil	Nil	Nil

Table 4:17 Microbial growth for Labaneh sample stored at 5 ˚C fortified with 1% (w/w) OLE, expressed as CFU

Time	Y&M	STAPH	F.COLI	T.COLI
0Week	Nil	Nil	Nil	Nil
1Week	Nil	Nil	Nil	Nil
2Weeks	Nil	Nil	Nil	Nil
3Weeks	Nil	Nil	Nil	Nil
4Week	Nil	Nil	Nil	Nil
8Weeks	Nil	Nil	Nil	Nil

The above results presented the using of 1% of OLE. It showed that the same effects on microbial growth as appeared in 0.6% addition,which confirmed the effect of OLE as a successful antimicrobial agent. This result comes in accordance with Ali M. Ahmed et al,

2014.Who reported a high antimicrobial effect for OLE on preserving shrimp (Penaeussemisulcatus) products agents microbial growth when using a high concentration of OLE reached up to 2%. This high concentration was due to a high protein and aqueous matrix (fish), while stronger and more stable protein as, in case of cheese (high impact matrix), much lower concentration were effective.

4.4.2. The Sensorial evaluation of Labaneh Products:

The sensorial evaluation of fortifying Labaneh with OLE was studied enable to introduce better understanding for the ability of such fortification.

The finite amount of fortification (0.2%OLE) was detected easily by panelist either by researcher or the market group. The sensorial evaluation demonstrated highly inacceptance for Labaneh color for such tiny addition of OLE, never mind fortification with elevated concentration.

Even more, the rejection of the sample was not limited to the color itself, but also expressed by taste and flavor of the sample.

This finding expected as labaneh is very white dairy product and accept no alteration in its general form.

Table 4:18 Sensorial evaluation of Labaneh samples fortified with different concentrations of OLE

	Ranking (% of participant)								
Characteristics	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Standard sample without fortification									
Taste	0	0	0	0	0	0	0	20	80
Flavor	0	0	0	0	0	0	10	10	80
Color	0	0	0	0	0	0	10	0	90
Texture	0	0	0	0	0	30	0	10	60
Labaneh sample fortified with 0.2% OLE									
Taste	0	0	60	10	20	10	0	0	0
Flavor	0	0	0	10	50	40	0	0	0
Color	0	60	10	20	10	0	0	0	0
Texture	0	0	0	40	30	30	0	0	0
Labaneh sample fortified with 0.4% OLE									
Taste	0	60	20	20	0	0	0	0	0
Flavor	0	40	30	10	20	0	0	0	0

Color	50	30	20	0	0	0	0	0	0
Texture	0	0	0	50	20	30	0	0	0
Labaneh sample fortified with 0.6% OLE									
Taste	70	20	10	0	0	0	0	0	0
Flavor	0	70	30	0	0	0	0	0	0
Color	90	10	0	0	0	0	0	0	0
Texture	0	0	0	60	40	0	0	0	0
Labaneh sample fortified with 1% OLE									
Taste	90	10	0	0	0	0	0	0	0
Flavor	90	10	0	0	0	0	0	0	0
Color	100	0	0	0	0	0	0	0	0
Texture	0	0	50	40	10	0	0	0	0

In form of texture it is obvious that labaneh fortified with OLE, regardless the concentration, demonstrated more loos texture and watery.

This results justified due to the ability of curd to merge with total phenols and in the presence of water in the Labaneh matrix, the water increased dissolving the total protein which became more watery and less rigidity.

4.4.3. The shelf life of Labaneh Products fortified with OLE:

According to the microbial results obtained and the observation of the samples after storage at 5 °C for 2 months, it is clear that the addition of OLE, mainly 0.6 and 1% highly helped in increasing product shelflife. But the same time the product color was completely became darker due to the darkish color of OLE. It is clear from the studied samples that the samples developed no oxidized color, althoughthe sample was very dark in comparison to the non fortified sample. This is due to the fact that shelf life and keeping quality was improved with the addition of OLE due to the effect of phenolic compounds that retard the oxidation, Visioli et al., (2002). And the OLE antimicrobial activity. Owen et al. (2000).

4.5. Olive Leaf Extract Analyses

In order to obtain OLE, powdered olive leaves were mixed with deionized water (PH=3)at 40ºC for 4hrs.Then evaporated until total solid reached 42.The obtained crude extractwas then ready to be used in further analyses.

4.5.1. Phenolic Compounds in Olive Leaf Extract:

Oleuropein is the major phenolic compound that contributes to total antioxidant capacity of OLE. Efficiency of the extraction process depends on the amount of oleuropein obtained from the process, since it increases the bioactivity of olive leaf extract. The qualitative and quantitative determination of oleuropein can be achieved by performing HPLC analysis.

The concentration of oleuropein in olive leaves extract was determined according to the following formula:

$(A_{sample}/A_{std.})* C_{std.}/C_{sam.}$

Where $A_{sample}$is the area of oleuopein peak in sample solution.

A_{std} Is the area of oleuopein peak in standard solution

$C_{std.}$ is the concentration of oleuopein in standard solution.

$C_{sam.}$is the concentration of oleuopein in sample solution.

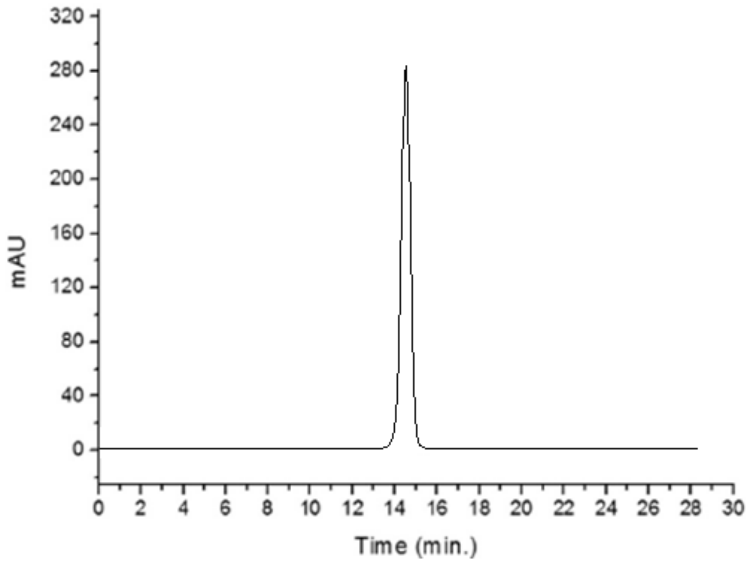

Fig 4:1HplcChromatogram of oleuropein Standard

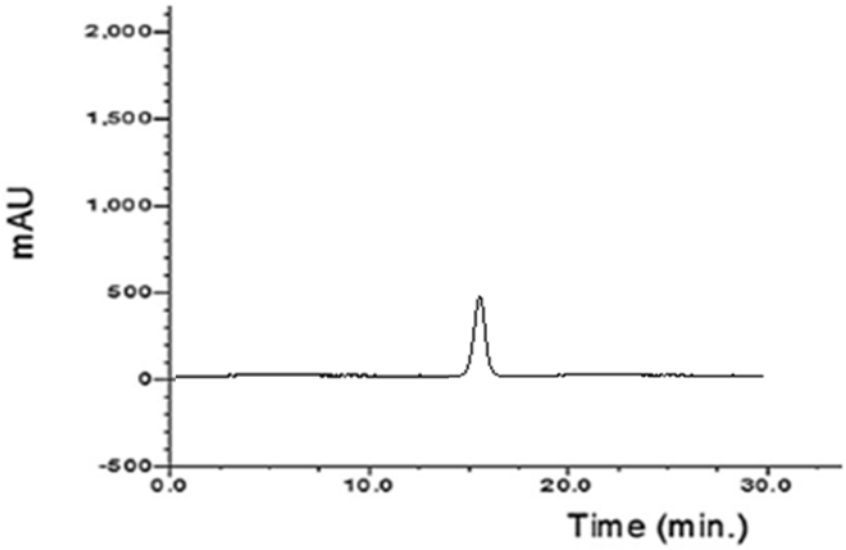

Fig 4:2 Hplc Chromatogram of Oleuropein

Chromatogram of oleuropein Standard and Sample ofolive leaves extract; other peaks thatappear in the chromatogram are for other compoundspresent in the olive leaves. Mobile phase: acetonitrile/phosphate buffer pH 3.0 (20:80, v/v), flow rate 1.0 mL/min, Injection volume 20 µL. Column: C18, 5 mm (5 mm,150 x4.6mminner diameter), UV detection: 280 nm.

Chapter Five

5. Conclusion and Recommendation

This chapter will discuss the conclusions drown in this investigation which is mainly in the field of OLE effect as a natural additive in dairy products.

This chapter will show a generated recommendation drown from the needed issues need to be covered in continuing the same field of study.

5.1. Conclusion

The main conclusions obtained in this investigation are:

- OLE has a considerable effect as antimicrobial agent when it's fortified in cheese, labaneh, and pudding.
- OLE has a considerable effect as antioxidant agent as revealed in product shelf-life
- OLE enhanced the sensorial characteristics of cheese and pudding positively.
- OLE has a bad effect on the sensorial evaluation of Labaneh.
- OLE has enhanced the studied dairy product shelf-life through its effect positively on microbial growth, antioxidant effect, and sensorial evaluation.
- The remarkable positive effect of OLE addition starting from 0.4% addition.
- OLE played a positive effect on developing the texture and consistency of the pudding product.
- OLE developed new preferable features in cheese which is increasing the elasticity.
- The new invented method of OLE extraction is introducing higher amount of olieuropien in comparison to the old traditional extraction method.

5.2. Recommendation

The results and notifications generated in this field forcing us to recommend a continual study including other dairy products. After completion of this study the following recommendations could be interested for the future work.

1. It is highly needed to study exclusively the antioxidant effect of OLE from chemical; point of view.

2. It is highly recommended to incorporate a bleaching agent for developing higher acceptance of OLE addition in white dairy product and mainly labaneh.

3. Investigation of the effect of OLE on food stuff other than dairy products

4. Preparation of a nutritive supplements as compressed tablets.

5.Studying applications for OLE other than food products such as cosmetics or protection cream

6. Investigating the health benefits of OLEas food additive on the human

7. Studying the effects of added OLEon the beneficial normal pro-biotic bacteria on the gastro-intestinal track.

References:

1. Albi, T. Guinda, A. Lanzón, A. 2001 ., Procedimiento de Obtención y eterminación de ÁcidosTerpénicos de la Hoja del Olivo OleaEuropaea. *Grasas y Aceites*, 52, 275-278
2. Ahmed, Ali, Rabii, Nancy, and Abolghait, Said. 2014., Effect of olive (Oleaeuropaea L.) leaves extract in raw peeled undeveined shrimp (Penaeussemisulcatus). International Journal of Veterinary Science and Medicine, .2, 53–56
3. Al-Qarawi A., Al-Damegh M., and El Mougy S. 2002., Effect of freeze dried extract of Oleaeuropaea on pituitary-thyroid axis in rats, Phytotherapy Research, 16. 286-287
4. Amiot, M, Fleuriet A, and Macheix J, 1989., Accumulation of oleuropein derivatives during olive maturation, Photochemistry, 28(1), 67-69.
5. Benvente O, Castillo J, Ortuno J, and Del Rio A, 2002., Antioxidant activity of phenolicsextracted from Oleaeuropea leaves, Food chemistry, 68. 457-462,
6. Bisignano G, Tomaino A, Cascio R, Crisafi G, Uncella N, and Saija A, 1999., On the in vitro antimicrobial activity of oleuropein and hydroxytyrosol, Pharm. Pharmacol, 51. 971-974,
7. Block, G, Patterson, B, and Subar, A, 1992., Fruit, vegetables, and cancer prevention: A review of the epidemiological evidence. Nutr. Cancer 18: 1-29.
8. Briante R, Cara F, Frebbraio ., Patumi M, and Nucci R, 2002., Biotransformation on OleaEuropea leaf extracts, Journal of Biotechnology, 93, 109-119,
9. Carluccio M, Siculella L, Ancora M, Massaro M, Scoditti E, Storelli C, Visioli F, Distante A, and DeCaterina R., 2003., Olive oil and red wine antioxidant polyphenols inhibit endothelial activation: antiatherogenic properties of

Mediterranean diet phytochemicals, Arterioscler ThrombVasc Biol, 23(4):622–629

10. David A, Saleh S, Antonio S, Javier M, and Alberto F, 2008., Identification of phenolic compounds, Agro Food industry hi-tech, 19, n6,
11. De Leonardis A, Aretini A, Alfano G, Macciola V, and Ranalli G, 2008., Isolation of hydroxytyrosol-rich extract from olive leaves (OleaEuropea) and evaluation of its antioxidant properties and bioactivity, European Food Research and Technology, 226(4), 653-659
12. Hansen, K, Adsersen, A, Christensen, BS, Brooegger, S, Rosendal, JS, Nyman U, and Wagner, SU. 1996., Isolation of an angiotensin converting enzyme (ACE) inhibitor from Oleaeuropaea and Olealancea. Phytomedicine, 2, 319 324
13. Alyateem, Hiba, 2014. Extraction of oleuropein from Palestinian olive leaf by using simple extraction methods and apply the extract in cosmetic products. M.Sc. Palestien: Al-Quds University.
14. Humeid, H.A, Tukan, S.K, and Yamani, M.I, 1990., In bag steaming of white brined cheese as a method of preservation. Michwissen chaft, 45: 513-516.
15. Goldberg, I, 1996. Functional foods. Designer foods, pharma food, nutraceuticals. 1st ed.Chapman and Hall. Londres, Gran Bretaña
16. Le Tutour B. and Guedon D. Antioxidative activities of Oleaeuropea leaves and related phenolic compounds phytochemistry, 31:1173-1178, 1992.4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced lung tumorigenesis in female A/J mice. *Cancer*
17. Aytul, K, 2010.Antimicrobial and Antioxidant Activities of Olive Leaf Extract and its food applications. M.Sc. Izmir, İzmir Institute of Technology.
18. Lee, O.H, and Lee, B.Y. 2010., Antioxidant and Antimicrobial Activities of Individual and Combined Phenolics in *Oleaeuropea*Leaf Extract. *Bio. Tech.* 101, 3751- 3754.
19. Lee-Huang, Manna, C, Galletti, P, Cucciolla, V, Montedoro, G, and Zappia V. 2003., Anti-HIV activity of olive leaf extract (OLE) and modulation of host cell gene expression by HIV-1infection and OLE treatment, Biochemical and Biophysical Research Communications 307 1029 103

20. Markin, D. Duek, L,and Berdicevsky, I, 2003., In vitro Antimicrobial Activity of Olive Leaves. *Mycoses***,** 46, 132-136.
21. Medina E, A de Castro, C Romero and M Brenes. 2006., Comparison of the concentrations of phenolic compounds in olive oils and other plant oils, correlation with antimicrobial activity. J Agric Food Chem 54: 4954-4961.
22. Messer, J.W, Behirey, H.M. and Leudecke L.O., 1985., Standard Methods for Examination of Dairy Products. 15th Edn., American Public Health Association, Washington, DC., ISBN 08-75531326, pp: 412..
23. Micol V, Caturla N, Perez-Fons L, Mas V. and Perez L, A, 2005., The olive leaf extract exhibits antiviral activity against viral haemorrhagic septicaemiarhabdo virus (VHSV)" *Antiviral Research,* 66. 129-136.,
24. Marhamatizadeh, M, Ehsandoost, El Paria, G Mohammad and Mohaghegh, D. 2013., Effect of Olive Leaf Extract on Growth and Viability of *Lactobacillus acidophilus* and *Bifidobacteriumbifidum*for Production of Probiotic Milk and Yoghurt . International Journal of Farming and Allied Sciences, -2-17/572-578.
25. Nagayama K, Y Iwamura, T Shibata, I Hirayama and T Nakamura. 2002. Bacterial activity of phlorotannins from the brown alga Eckloniakurome. J AntimicrobChemother 50: 889-893.
26. Owen RW· Haubner R, Würtele G, Hull E, Spiegelhalder B, and Bartsch H. 2004., Olives and olive oil in cancer prevention,13(4):319-26
27. Pereira, A.P.Ferreira, I.C. Marcelino, F.Valentão, P. Paula, B. Seabra, R. Estevinho, L. Bento, A. And Pereira, J.A. 2007., Phenolic Compounds and Antimicrobial Activity of Olive (Oleaeuropaea L. Cv. Cobrançosa) Leaves. Molecules, 12, 1153–1162.
28. Pooley R., and Peterson L., 1997.Mechanisms of microbial susceptibility and resistance to antimicrobial agents, In the Biologic and Clinical Basis of Infectious Diseases, 5th Edition. Editors ST, Shulman JP, Phair LR, Peterson JR. Philadelphia: W.B. Saunders Company, pp. 550
29. Syed H. 2010., Oleuropein in Olive and its Phamacological effects. Scientia Pharmaceutical, pp.133-154,.

30. Ryan and Robards, *1998*. Phenolic compounds in olives. *Analyst, May, Vol. 123 (31R–44R)*
31. Shtukatur I. 2003.The technology of cracker production to improve the condition of sick people. Patent N° WO 2004080203-A2,.
32. Stueckler F., Natural substance based agent. Patent N° WO 9948386-A1, 1998.
33. Somova L., Shode F., Ramnanan P., and Nadar A., 2003. Antihy pertensive, anti atherosclerotic and antioxidant activity of tripenoids isolated from Oleaeuropaea, subspecies Africana leaves.J .Ethnopharmacol, 84(2-3)299,
34. Shtukatur I, 2003.The technology of cracker production to improve the condition of sick people. Patent N° WO 2004080203-A2,.
35. Somova L., Shode F., Ramnanan P., and Nadar A., 2003. Antih ypertensive, anti atherosclerotic and antioxidant activity of tripenoids isolated from Oleaeuropaea, subspecies Africana leaves. J .Ethnopharmacol, 84(2-3)299
36. Syed H. 2010., Oleuropein in Olive and its Phamacological effects. Scientia Pharmaceutical, pp.133-154.
37. Visioli F., Bellomo G., and Galli C. 1998. Free radical-scavenging properties of olive oil polyphenols. Biochem. Biophys. Res. Commun., 247, 60-64
38. Visioli F., Caruso D., Galli C., Viappiani S., Galli G., and Sala A., 2000. Olive oil rich in natural catecholic phenols decrease isoprostane excretion in humans. BiochemBiophys Res Commun. 278: 797–799.
39. Vissers MN, Zock PL, Roodenburg AJ, Leenen R, and Katan MB. 2002. Olive Oil Phenols Are Absorbed in Humans, The American Society for Nutritional Sciences. J Nutr.;132(3):409-17
40. Wisselink H., Weusthuis R., Eggink G., Hugenholtz J., and Grobben GJ., 2002. Mannitol production by lactic bacteria: a review. Int. Dairy J., 12,151-161,
41. Yamani, M. I., Humeid, M. A. and Tukan, S. 1987. Comparison of Keeping Ability of Nabulsi Boiled White Cheese Filled in Plastic Pouches Using Cold and Hot Brine. Dirasat. 14: 179-186.

استخدام مستخلص ورق الزيتون في زيادة فعالية منتجات الالبان

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الملخص:

جمعت أوراق الزيتون الخضراء من أشجار النوع النبالي البلدي من عدة أجزاء من الضفة الغربية, وتم تجفيفها على درجة حرارة الغرفة . تعرضت العينات لطرق استخلاص مائي مختلفة وهي:

1) الاستخلاص بالماء (النقع) بالطريقة التقليدية (القديمة)
2) وبواسطة طريقة جديدة تم ابتكارها تعتمد على الاستخلاص تحت تاثير الضغط وضبط الحرارة

ثم استخدمت أعلى كمية من أوليوروبين التي تم استخلاصها بواسطة الطريقتين تحليلها بواسطة جهاز HPLC في التطبيق على منتجات الالبان (الجبنة البيضاء النابلسية , البودنج واللبنة) بتراكيز مختلفة (0.2٪ و 0.4٪ 0.6٪ و 1٪).

وتعرضت المنتجات المضاف اليها مستخلص ورق الزيتون لعدة اختبارات:الفحوصات الميكروبية والحسية فضلا عن اختبارات مدة الصلاحية بالاعتماد على المواصفات الفلسطينية لهذه المنتجات . واظهرت النتائج تاثير واضح عند استخدام التركيز المرتفع لمستخلص ورق الزيتون على النتائج الميكروبية لمنتجات الالبان التي تم دراستها , وبدا التاثير واضح على المستوى الحسي لبعض المنتجات عند التركيز الاقل (0.4%) من المستخلص .

وكان استخدام التركيز 0.6% و1% من مستخلص ورق الزيتون له اعلى فعالية في تخفيض العدد الميكروبي طوال مدة الصلاحية .