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*International Journal of Food Engineering Research (IJFER) is peer-reviewed journal which provides a platform for publication of original scientific research and applied practice studies. Positioned as a vehicle for academics and practitioners to share field research, the journal aims to appeal to both researchers and academicians.*

## Aims and Scope

International Journal of Food Engineering Research (IJFER) is an international, peer-reviewed journal devoted to the publication of high quality original studies and reviews concerning a broad and comprehensive view of fundamental and applied research in food science&technology and their related subjects as nutrition, agriculture, food safety, food originated diseases and economic aspects.

IJFER is an international periodical published twice a year (April and October). The journal is published in both print and electronic format.

# *From The Editor*

*International Journal of Food Engineering Research (IJFER) has been publishing by Istanbul Aydın University Faculty of Engineering Department of Food Engineering since 2015. The journal covers wide ranges of area such as Food Processing, Food Preservation, Food Microbiology, Food Chemistry, Biotechnology, Nanotechnology, Novel Technologies, Food Safety, Food Security, Food Quality and their related subjects as nutrition, food and health, agriculture, economic aspects and sustainability in food production.*

*Food Engineering is getting more and more attention because it is directly related to human health. While the food and drinks we eat help to protect our health, on the other hand, improper conditions during the conversion of the raw material to the product, the use of poor quality raw materials, and the employees not working under hygienic conditions can cause the food harmful to health. Our aim in this journal is to include the recent research and reviews on food and beverages from field to fork. Articles submitted to the journal are accepted for publication after being reviewed by expert referees.*

*In the following years, the journal will include scientific activities such as symposiums, congresses, conferences and workshops held in the field of food science and technology, and information about the books published in this field. We hope that the journal will be a good resource for engineers, experts, researchers and students working in the food industry.*

***Prof. Dr. Z. Dilek Heperkan***
***Editor***

*International Journal of Food Engineering Research (IJFER)*

## *Contents*

### Research Articles



### Review Articles

## *DOI Numbers*

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# USE OF DIFFERENT ESSENTIAL OILS IN CONCENTRATED YOGURT AS NATURAL PRESERVATIVE*

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## ABSTRACT

The main method of producing labneh consists of straining whole milk yogurt in a cheesecloth bag to the desired total solid level, it is a critical step in labneh manufacturing, due to the sanitary problems usually associated with the cloth bags used, which increases microbial contamination. In this study, essential oils (EOs) are used to increase the shelf life of labneh from 4 weeks to at least 6 weeks with decrease in the concentration of synthetic antimicrobial agent used. Measurement of the antimicrobial activity of EOs is done using total plate count method, on mold, yeast, *Staphylococcus aureus*, coliforms, and *Escherichia coli* O157:H7 .

The EOs used in this study are namely clove, rosemary, sweet almond oil. They were added to labneh without any synthetic preservative. EOs were added at

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concentrations 600 µl/kg without addition of the synthetic preservative. Total solids of labneh sample, treated with EOs, were only slightly affected. EOs affect the pH. In the presence of synthetic preservative, in terms of influence a total bacterial viable count, the best EOs used were found to be clove and rosemary. The mold count for EOs, the best EOs used were found to be clove and rosemary. yeast decreased, where the best EOs were found to be cinnamon, clove, rosemary, sweet almond. However, clove, rosemary, sweet almond when added to labneh significantly decreased the growth of *S. aureus* and even better than positive control. However, for EOs used the best essential oil that significantly decreased the growth of *S. aureus* was found to be rosemary at concentration of 600 µl/kg. No coliform bacteria or *E. coli* were detected in the treated labneh as well as in the positive control. The most acceptable organoleptic properties of treated labneh were rosemary oils followed by sweet almond. Organoleptic properties in these groups were better than positive control. In this study, it can be concluded that the addition of rosemary and clove EOs at (600 µl/kg), could increase the shelf life of labneh for up to 6 weeks instead of 4 weeks.

**Keywords:** *Concentrated yogurt (Labneh), Essential oils, Dairy products.*

## INTRODUCTION

Many food products are perishable by nature and require protection from spoilage during their preparation, storage, and distribution to give them desired shelf life, especially dairy product. Food products can be subjected to contamination by bacteria and fungi. Many of these microorganisms can cause undesirable reactions that deteriorate flavor, odor, color, sensory and textural properties of food. Illness can be caused because of the consumption of foods contaminated with pathogens such as *Staphylococcus aureus*, *Escherichia coli* O157, *Salmonella*, Fecal coliform, total coliform, yeast, and mold. To prevent growth of spoilage and pathogenic microorganisms in foods, several preservation

techniques, such as heat treatment, salting, acidification, and drying have been used in the food industry **[1, 2]**. In addition, a chemical method can be used which involved the use of chemical preservatives and artificial antimicrobials to inactivate or inhibit growth of spoilage and pathogenic microorganisms **[3, 4].** Numerous efforts are conducted to find natural alternatives to prevent bacterial and fungal growth in foods. In recent years, because of the great consumer awareness and concern regarding synthetic chemical additives, foods preserved with natural additives have become very popular. To inhibit growth of undesirable microorganisms in food, the antimicrobials can be directly added into the product formulation, coated on its surface, or incorporated into the packaging material. Direct incorporation of active agents into food results in an immediate but short-term reduction of bacterial populations, while the antimicrobial films can maintain their activity for a long period of time **[5, 6].**

Natural antimicrobials are derived from animal, plant, and microbial sources. There is considerable potential for utilization of natural antimicrobials in food. However, methods and mechanisms of action, as well as the toxicological and sensory effects of natural antimicrobials, are not completely understood **[7, 8].** Main natural compounds are EOs derived from plants (e.g., cinnamon, clove, rosemary, sweet almond, sesame, wheat germ, sandalwood, basil, thyme, eucalyptus and oregano), enzymes obtained from animal sources (e.g., lysozyme, lactoferrin), bacteriocins from microbial sources (nisin, natamycin), organic acids (e.g., sorbic, propionic, citric acid, benzoic), and naturally occurring polymers (chitosan).

Most plant EOs are gaining a wide interest in the food industry for their potential as decontaminating agents, as they are Generally Recognized as Safe (GRAS). The active components are commonly found in the essential oil fractions and it is well established that most of them have a wide spectrum of antimicrobial activity, against food-borne pathogens and spoilage bacteria **[9, 10].**

The antimicrobial activity of plant EOs is due to their chemical structure, to the presence of hydrophilic functional groups, such as hydroxyl groups of phenolic components and/or lipophilicity of some essential oil components **[11].** Usually, the compounds with phenolic groups such as oils of clove, oregano, rosemary, thyme, sage, and vanillin are the most effective **[12].** They are more inhibitory against gram-positive than gram-negative bacteria **[13, 14].**

Many reviews focus on the use of natural compounds to control microbiological and physicochemical shelf life of main food categories, such as meat, fish, dairy products, minimally processed fruit and vegetables and cereal-based foods. The information is mostly based on case-studies dealing with application of active compounds to prevent microbial proliferation occurring in packaged food during storage.

EOs are very interesting natural plant products and among other qualities, they possess various biological properties. The term "biological" comprises all activities that these mixtures of volatile compounds (mainly mono-and sesquiterpenoids, benzenoids, phenylpropanoids, etc.) exert on humans, animals, and other plants **[7, 8].**

Milk the main component of labneh, a concentrated fermented yogurt, is a good media for many bacterial growths including pathogens. Labneh is a semisolid food that results from the concentration of yogurt using different methods. The most important is the use of cloth bags and draining the yogurt for 14 hours. The total solid of the resulting labneh is approximately 23 g/100g and the product has a cream white color and a flavor that is slightly acidic, the texture is soft and smooth. The high microbial load of labneh, coupled with the packaging and storage conditions, result in the formation of off-flavors and undesirable physicochemical changes that eventually lead to rejection of the product **[15].**

One of the most accepted methods to extend the shelf life of perishable food products is using bio-preservatives **[7, 16].**

Concentrated yogurt is popularly known as labneh in the Middle East or as strained yogurt in Greece, and the rest of Europe, or as süzme yogurt in Turkey. Labneh is a semisolid fermented dairy food produced by removing part of the whey from yogurt to reach total solid levels between 23 and 25 g/100 g **[17].**

Labneh preparation is carried out as follows: Fresh cow's milk (3% fat) was heated at 90°C for 20 minutes, cooled to 45°C and then inoculated with 2% of the yogurt starter culture (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*). The milk was agitated, dispensed in glass containers, and incubated at 40°C for 3 hours until it was completely coagulated. The resultant coagulant was mixed thoroughly with 0.5% sodium chloride. The mixtures were then put into cheesecloth bags, which were hung in the refrigerator room at 5 ± 1°C for 18 hours, to allow drainage of the whey **[18, 19].**

EOs are not simple compounds or even simple mixtures of several individual compounds. They may contain up to approximately 100 components, although many contain about 20 to 60. The compounds found in EOs are from a variety of chemical classes, predominantly terpenes, but phenylpropanoids and other compounds also occur although at a lesser frequency and often, but not always, in smaller proportions. They are all hydrocarbons and their oxygenated derivatives, and they may also contain nitrogen or sulfur. They are generally low-molecular-weight compounds with limited solubility in water **[20].**

The classification and nomenclature of essential oil compounds are complicated by the fact that many were isolated and studied before the instigation of systematic chemical nomenclature. Consequently, many are known by nonsystematic or trivial or common names. These are sometimes but not always

based on their source, such as eucalyptol, limonene, pinene and thymol, names which hint at historical botanical origins of these compounds.

In terms of shedding light on their chemistry, the long history and widespread use of these nonsystematic names further obfuscates the chemical nature and characteristics of EOs and their components **[21].**

EOs are a group of terpenoids, sesquiterpenes and possibly diterpenes with different groups of aliphatic hydrocarbons, acids, alcohols, aldehydes, acyclic esters, or lactones **[22].** EOs and other plant extracts are principally responsible for antimicrobial activities in plants, herbs, and spices. These extracts can be obtained from plants and spices by various methods, such as steam, cold, dry, and vacuum distillation. These plant compounds, including glucosides, saponins, tannins, alkaloids, EOs, organic acids and others, are present as parts of the original plant defense system against microbial infection **[23, 24].** Generally, phenolic compounds of EOs such as citrus oils extracted from lemon, olive oil (oleuropein) and tea-tree oil (terpenoids), orange and bergamot have broader antimicrobial effects and are not categorized as spices. Meanwhile, there are increasing reports of nonphenolic compounds of oils, which are effective against both gram-positive and gram-negative groups of bacteria, from oregano, clove, cinnamon, citral, garlic, coriander, rosemary, parsley, lemongrass, purple (cultivar Ison) and bronze (cultivar Carlos) muscadine seeds and sage **[25, 26, 27].** Little information is available on interaction among constituents in essential oil sweet almond and the effects they have on antimicrobial activity.

Phenolic components are responsible for antimicrobial action and other constituents are believed to have little activity. Dependability of EOs as antimicrobials could be improved if their content of active agents should be standardized by distillation **[28].**

## MATERIALS AND METHOD

### Materials

EOs such as Clove (*Syzygiumarom aticum*) oil, Sweet almond (*Prunus dulcis*) oil, Cedarwood (*Cedrus spp*) oil and Rosemary (*Rosmarinus officinalis*) oil used in this study were obtained from Al-Jibrini for food industries (Hebron, West Bank, Palestine).

All EOs were stored at cold temperature 5°C until analysis. Labne used in the analysis prepared from fresh and pasteurized milk and stored +4°C until analysis.

Ethanol, Water, Microbiological media (Plate count agar for the detection viable bacterial growth in labneh, Violet Red Bile Agar recommended for the detection of coliforms in labneh, Eosin Methylene Blue for the detection of E. Coli in labneh, Oxytetra Glucose Yeast Agar base for the detection of yeast and mold in labneh, Baird–Parker agar for the detection of Staphylococcus aureus in labneh), peptone water. All these used in this study were obtained from Himedia Laboratories (Mumbai – India).

### Method

### Antimicrobial activities of EOs

The antimicrobial activity of EOs will be evaluated against major microorganisms that can be present in labneh such as Coliforms*, Escherichia coli O157:H7*, yeast, mold, *Staphylococcus aureus* and total count bacteria.

Experiments will involve the evaluation of the effect of the addition of EOs each type separately, rosemary oil, sweet almond oil, cedarwood oil, clove oil at different concentrations, 600 µl/kg, on the microorganisms that present in labneh.

**Addition of EOs to labneh**

Addition of one of the EOs: rosemary oil, sweet almond oil, clove separately, to one kilogram of labneh sample at concentrations 600 µl/kg, without addition of synthetic preservative (potassium sorbate). The resulting mixture is then mixed for 15 minutes and distributed to six packages of 200 mg and stored in fridge at 5°C for 6 weeks.

**Chemical analysis**

The methodology reported by Ling (1963) **[29]** was used to determine the total solid content, and pH of the different labneh samples.

**Microbiological analysis**

Evaluated antibacterial activity and properties against major labneh borne bacteria such as, coliforms, *Escherichia coli O157:H7*, yeast, mold, *Staphylococcus aureus* and total aerobic count bacteria by plate count method, (pouring plate method) is used for counting microorganisms in labneh.

1 g sample of labneh was diluted in 9 ml of peptone water yielding a $10^{-1}$ dilution. Serial dilutions were subsequently prepared and viable numbers were enumerated using the pour plate technique. Total viable counts (TVC) were determined according to Klose (1968) **[30],** the agar plates were incubated at 30°C for 72 hours. Mold and yeast counts were determined according to Harrigan and McConce (1966) **[31],** while *coliform* bacteria were enumerated using the method described by the American Public Health Association (1978) **[32].** The colony forming units (CFU) were converted to log10 and the results are reported as the average from three replicates, each colony can be counted and represents a single cell in the labneh. When labneh sample is mixed with liquefied agar, then must be used dilution to obtain accurate quantitative analyses of cell number. In

microbiological tests, every plate was repeated three times for each type of bacteria, and calculates the mean, then the standard deviation.

**Organoleptic properties**

All labneh samples were sensory evaluated for flavor (50 points), body and texture (40 points), and appearance (10 points) according to Keating and Rand-white (1990) **[33]**. All samples were evaluated by eight people, specialists in food science, and rated by percentage.

## RESULT AND DISCUSSION

**Effect of EOs in labneh on total viable counts of bacteria**

Different types of EOs such as sweet almond, clove, eucalyptus, rosemary oil, were used as preservatives of labneh sample and compared to positive control (potassium sorbate, 300 ppm) which used in labneh manufacturing in Palestine and compared to negative control (no preservatives added). Some EOs such as clove and rosemary showed a clear effect with reduction in bacterial, mold and yeast count throughout the six weeks, and others such as sweet almond did not show obvious effect.

TVC decreased in the presence of EOs compared with the positive control samples. This activity is due to the antibacterial effect of EOs, during storage period. On the other hand, total bacterial viable count reached $13.00\times10^{1}$ CFU/g in the positive control sample, while in the best EOs clove, rosemary the total bacterial viable count, at 600 µl/kg oil concentration the TVC reached $12.00\times10^{1}$ CFU/g in rosemary labneh. This activity is due to the antibacterial effect of EOs, during per storage period.

Quality and shelf life of labneh are evaluated with mold and yeast counts, so molds were detected at small number in labneh containing clove oil, rosemary oil

throughout the storage period. At the end of the storage period molds number reached $7.00\times10^1$ CFU/g in positive control sample, at 600 µl/kg oil concentration the best EOs were clove, rosemary, mold in treated labneh with clove labneh mold number reached $6.00\times 10^1$ CFU/g, and in rosemary.

Yeasts were detected at small number in labneh containing rosemary throughout and at the end of the storage period, at least similar to positive control effect. At 600 µl/kg oil concentration the best EOs clove, yeast in treated labneh with clove reached $5\times10^1$ CFU/g, followed by rosemary yeast number reached $6.00\times10^1$ CFU/g. In Sweet almond there was no obvious effect on yeast content.

The results obtained for *Staphylococcus aureus* indicated that bacteria detected at small number compared with positive control, in labneh containing rosemary throughout and at the of end the storage period. At the end of the storage period *S. aureus* number reached $8.00 \times 10^1$ CFU/g in positive control sample, at 600 µl/kg oil concentration the best essential oil is rosemary, *S. aureus* in treated labneh reached $6\times10^1$ CFU/g, followed by clove $8\times10^1$ CFU/g. While in labneh containing sweet almond were not show obvious effect.

Both coliform and *E. coli* were not detected in any of the labneh prepared by addition of the respective EOs. This effect may be attributed to an effect of active compounds in the EOs; Burt (2004) **[7]** reported that EOs contain phenolic compounds that are primarily responsible for their antimicrobial properties. Our results indicated that these bacteria show a few inhibits at low concentrations of the different EOs, while an increase in the oil concentrations leads to decreases in bacterial, yeast and mold counts.

Clove oil and rosemary oil has good antiseptic, antibacterial and antifungal properties, because contain phenols and monoterpene, alcohols, monoterpene, aldehydes esters, lactones and phenylpropenes **[20].**

The phenylpropenes constitute a relatively small part of EOs, and those that have been most thoroughly studied are eugenol, isoeugenol, vanillin, safrole, and cinnamaldehyde. The comparison of the molecules that are chemically similar to eugenol and isoeugenol indicated that the free hydroxyl groups are important for their activity against bacteria **[34].** Furthermore, the antimicrobial activity of phenylpropenes depends on the kind and number of substituents on the aromatic ring, selected microbial strains, and the experimental test parameter such as choice of growth medium, temperature **[35].**

Clove oil contains 80% of eugenol, 4.5% in cinnamon oil and it's the bioactive compound that responsible for antibacterial and antifungal effect. And its antimicrobial activity is linked to its ability to permeabilize the cell membrane and interact with proteins. Eugenol's action on membranes occurs mainly by a non-specific permeabilization **[36, 37].**

Eugenol induced minor changes in the fatty acid profile of *Pseudomonas fluorescens*, *E. coli*, *Brochotrix thermosphacta*, *S. enterica*, and *S. aureus*, and cell damages to *E. coli* and *B. thermosphacta* cells **[38, 39].** Consistent with this, eugenol has proven to inhibit the activity of the following enzymes: ATPase, histidine decarboxylase, amylase, and protease. Inhibition of the ATPase may be important for cell killing at high Eugenol concentrations because energy generation needed for cell recovery is impaired **[36].** The antifungal mode of action of eugenol needs further investigation, but it is known to depend on cell proliferation **[40].**

**Total viable counts of labneh at 600 µl/kg oil concentration**

When comparing the positive control and negative control, with labneh samples at a concentration of 600 µl/kg EOs, sweet almond oil did not show obvious effect on the labneh sample compared to positive control (Table 1). Concerning clove

oil and rosemary results showed that there was relative obvious decrease in bacterial count, because the bacteria count was a slightly higher than positive control especially in the last week and because bacteria did not multiply very quickly compared with samples without preservatives because of essential oil (Table 1).

**Table 1.** Total viable counts of labneh during 6 weeks at 600 µl/kg oil concentration

| | Total Viable Counts of Labneh | | | | | |
|---|---|---|---|---|---|---|
| | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 |
| Sweet almond oil | 9.00 ± 2.52 | 11.00 ± 1.00 | 13.00 ± 3.51 | 19.00 ± 2.52 | 23.00 ± 2.52 | 27.00 ± 2.08 |
| Clove oil | 8.00 ± 1.00 | 8.00 ± 0.58 | 9.00 ± 0.58 | 10.00 ± 2.00 | 12.00 ± 1.53 | 16.00 ± 1.15 |
| Rosemary oil | 9.00 ± 2.00 | 6.00 ± 2.08 | 10.00 ± 0.58 | 10.00 ± 1.15 | 10.00 ± 4.93 | 12.00 ± 1.53 |
| Control 300 ppm potassium sorbate | 8.00 ± 2.00 | 9.00 ± 0.58 | 9.00 ± 1.00 | 8.00 ± 0.58 | 9.00 ± 0.58 | 13.00 ± 2.52 |
| Control without preservatives | 17.00 ± 3.61 | 23.00 ± 3.79 | 37.00 ± 6.00 | 50.00 ± 0.00 | 100.00 ± 0.00 | 100.00 ± 0.00 |

**Mold content in labneh at 600 µl/kg oil concentration**

When comparing the positive control and negative control, with labneh samples at a concentration of 600 µl/kg EOs, sweet almond oil, did not show obvious effect on the labneh sample compared to positive control. The mold count was less than negative control (Table 2).

When rosemary oil and clove oil was used and compared with the positive control, results showed that there was relative obvious decrease in mold count, mold content approximately constant from the first week until the last week as well mold content in the last week less than positive control, this is an evidence of the effect of oil throughout the six weeks (Table 2).

It is noteworthy to mention that all the EOs at this concentration showed mold count less than the negative control throughout the six weeks. This is a promising result showing the effectiveness of EOs on the mold count when the absence of synthetic preservative (potassium sorbate) compared to that usually used for labneh preservation (300ppm). This showed the effect of EOs in labneh preservation.

When all EOs were compared with the positive control, the best essential oil was rosemary oil, followed by clove oil (Table 2).

**Table 2.** Mold content of labneh during 6 weeks at 600 µl/kg oil concentration

| | Mold Content of Labneh | | | | | |
|---|---|---|---|---|---|---|
| | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 |
| Sweet almond oil | 5.00 ± 1.00 | 8.00 ± 0.58 | 7.00 ± 2.52 | 16.00 ± 2.08 | 16.00 ± 5.86 | 18.00 ± 5.03 |
| Clove oil | 2.00 ± 0.58 | 2.00 ± 0.58 | 4.00 ± 1.00 | 5.00 ± 1.00 | 5.00 ± 0.00 | 6.00 ± 0.58 |
| Rosemary oil | 2.00 ± 1.00 | 2.00 ± 1.00 | 2.00 ± 1.53 | 5.00 ± 1.15 | 5.00 ± 1.53 | 5.00 ± 1.15 |
| Control 300 ppm potassium sorbate | 1.00 ± 0.58 | 1.00 ± 0.58 | 2.00 ± 0.58 | 3.00 ± 1.15 | 5.00 ± 1.53 | 7.00 ± 1.53 |
| Control without preservatives | 6.00 ± 1.53 | 8.00 ± 1.53 | 11.00 ± 1.00 | 21.00 ± 2.00 | 50.00 ±0.00 | 100.00 ± 0.00 |

**Yeast content in labneh at 600 µl/kg oil concentration**

When comparing the positive control and negative control, with labneh samples at a concentration of 600 µl/kg EOs, sweet almond oil did not show obvious effect on the labneh sample compared to positive control. The bacterial count was less than negative control (Table 3).

Concerning clove oil when compared with the positive results showed that there was obvious decrease in bacterial count, because bacteria count is a bit higher than positive control in the sixth week in the labneh sample, also multiplication of yeasts slow compared with normal multiplication due to the oil effect of, and the effect of oil similar to positive control effect until the end of period (Table 3).

It is noteworthy to mention that all the EOs at this concentration showed yeast count less than the negative control throughout the six weeks. This is a promising result showing the effectiveness of EOs on the yeast count when the absence of synthetic preservative (potassium sorbate) compared to that usually used for labneh preservation (300ppm). This showed the effect of EOs in labneh preservation.

When all EOs were compared with the positive control, the best essential oil was clove oil and followed by rosemary oil (Table 3).

**Table 3.** Yeast content of labneh during 6 weeks at 600 µl/kg oil concentration

| | Yeast Content of Labneh | | | | | |
|---|---|---|---|---|---|---|
| | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 |
| Sweet almond oil | 6.00 ± 0.00 | 6.00 ± 1.00 | 10.00 ± 1.00 | 16.00 ± 2.08 | 16.00 ± 3.79 | 17.00 ± 1.00 |
| Clove oil | 5.00 ± 0.58 | 6.00 ± 0.58 | 9.00 ± 0.58 | 16.00 ± 1.15 | 18.00 ± 0.58 | 22.00 ± 2.52 |
| Rosemary oil | 3.00 ± 0.58 | 2.00 ± 0.00 | 2.00 ± 0.58 | 2.00 ± 1.00 | 4.00 ± 0.58 | 5.00 ± 2.00 |
| Control 300 ppm potassium sorbate | 4.00 ± 1.15 | 2.00 ± 0.58 | 3.00 ± 1.00 | 4.00 ± 0.58 | 5.00 ± 0.58 | 6.00 ± 0.58 |
| Control without preservatives | 2.00 ± 0.58 | 2.00 ± 0.58 | 2.00 ± 0.00 | 4.00 ± 1.15 | 5.00 ± 1.53 | 5.00 ± 2.00 |

### ***S. aureus, E. coli* O157:H7 and coliform content in labneh at 600 µl/kg oil concentration**

When comparing the positive control and negative control, with a labneh sample at a concentration of 600 µl/kg EOs, sweet almond oil did not show obvious effect on the labneh sample compared to positive control. The bacterial count was less than negative control (Table 4).

Clove oil when compared with the positive control, results showed that there was relative obvious decrease in bacterial count, bacteria multiply slow compared to normal multiplication due to the oil effect, the effect was approximately similar to positive control effect (Table 4).

When rosemary oil was used results showed that there was relative obvious decrease in bacterial count, because bacteria multiply slowly compared to normal multiplication due to the oil effect, there was a difference in the number of bacteria from the first week until the sixth week, the number of bacteria decreases continuously until the end of the period (Table 4).

It is noteworthy to mention that all the EOs at this concentration showed that *S. aureus* count less than the negative control throughout the six weeks. This is a promising result showing the effectiveness of EOs on the *S. aureus* count when the absence of synthetic preservative (potassium sorbate) compared to that usually used for labneh preservation (300ppm). This showed the effect of EOs in labneh preservation. When all EOs were compared with the positive control, the best essential oil was rosemary oil, followed by clove oil (Table 4).

*E. coli* and coliform bacteria were not detected at 600 µl/kg oil concentration in all samples.

**Tables 4.** *Staphylococcus aureus* content of labneh during 6 weeks at 600 µl/kg oil concentration

| | *Staphylococcus aureus* Content of Labneh | | | | | |
|---|---|---|---|---|---|---|
| | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 |
| Sweet almond oil | 7.00 ± 1.00 | 6.00 ± 2.08 | 8.00 ± 1.15 | 9.00 ± 1.53 | 9.00 ± 1.53 | 12.00 ± 2.52 |
| Clove oil | 5.00 ± 0.58 | 4.00 ± 0.58 | 5.00 ± 1.00 | 7.00 ± 0.58 | 8.00 ± 0.58 | 8.00 ± 1.15 |
| Rosemary oil | 6.00 ± 0.58 | 6.00 ± 1.00 | 8.00 ± 0.58 | 5.00 ± 1.00 | 6.00 ± 1.00 | 6.00 ± 0.58 |
| Control 300 ppm potassium sorbate | 5.00 ± 0.58 | 3.00 ± 0.58 | 5.00 ± 0.58 | 4.00 ± 0.58 | 6.00 ± 1.53 | 8.00 ± 1.15 |
| Control without preservatives | 10.00 ± 1.53 | 14.00 ±1.15 | 15.00 ± 0.58 | 16.00 ± 2.00 | 32.00 ± 2.00 | 44.00 ± 6.00 |

**Effect of EOs on total solids content of labneh**

Table 5 show the changes in the total solids (TS) during storage. The TS content increased slightly in all treatments as the storage period increased. Clove labneh at week 6 had the highest TS content (600 µl/kg oil; 25.86%).

All samples were similar to the positive control at all concentrations in all weeks; the proportion of solids slightly increased during storage period; this increase could be described by moisture loss. Similarly, Ismail *et al*. (2006) **[41]** also reported that there were no observable differences in TS of labneh produced by addition of six different EOs. The data is also similar to literature **[42, 43, 44]** those of who reported that the TS of labneh ranged between 22 - 26%.

**Effect of EOs on pH**

Table 6 show the changes during storage in pH of labneh made with several types of EOs in the absence of synthetic preservative potassium sorbate. The change in pH is a very important factor since it affects the shelf life and the acceptability of labneh. Based on the results presented in tables, it is evident that pH values of the treated labneh decreased with an increase in the storage period. These results agreed with that obtained by Abbas and Osman (1998) **[45],** who reported that the pH decrease gradually during storage period and Titratable acidity increased gradually during storage period. Generally, in concentrated yogurt such as labneh, acidity and pH values vary depending on the starter culture and draining conditions. For this reason, in terms of acidity and pH there have been main different values in the literature **[46, 47, 48, 49].**

**Table 5.** Changes in the total solids (TS) content of labneh during storage at 600 µl/kg oil concentration

| | Total Solid Content of Labneh | | | | | |
|---|---|---|---|---|---|---|
| | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 |
| Sweet almond oil | 24.38 ± 0.23 | 24.47 ± 0.08 | 23.58 ± 0.07 | 23.62 ± 0.48 | 23.73 ± 0.22 | 23.83 ± 0.13 |
| Clove oil | 25.15 ± 0.34 | 25.24 ± 0.23 | 25.31 ± 0.13 | 25.59 ± 0.37 | 25.73 ± 0.29 | 25.86 ± 0.24 |
| Rosemary oil | 24.50 ± 0.15 | 24.56 ± 0.13 | 24.63 ± 0.04 | 24.78 ± 0.12 | 24.86 ± 0.09 | 24.84 ± 0.35 |
| Control 300 ppm potassium sorbate | 24.31 ± 0.17 | 24.49 ± 0.30 | 24.67 ± 0.16 | 24.81 ± 0.18 | 24.86 ± 0.14 | 24.91 ± 0.22 |
| Control without preservatives | 24.19 ± 0.06 | 24.32 ± 0.15 | 24.46 ± 0.12 | 24.58 ± 0.17 | 24.87 ± 0.30 | 25.12 ± 0.08 |

**Table 6.** Effect of some EOs on pH values of labneh during storage at 600 µl/kg oil concentration

| | pH Values of Labneh | | | | | |
|---|---|---|---|---|---|---|
| | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 |
| Sweet almond oil | 4.05 ± 0.01 | 3.94 ± 0.01 | 3.87 ± 0.01 | 3.83 ± 0.02 | 3.79 ± 0.01 | 3.70 ± 0.01 |
| Clove oil | 3.98 ± 0.01 | 3.95 ± 0.00 | 3.95 ± 0.02 | 3.93 ± 0.01 | 3.84 ± 0.01 | 3.80 ± 0.01 |
| Rosemary oil | 3.99 ± 0.01 | 3.95 ± 0.01 | 3.92 ± 0.01 | 3.90 ± 0.00 | 3.85 ± 0.00 | 3.80 ± 0.01 |
| Control 300 ppm potassium sorbate | 4.09 ± 0.00 | 4.05 ± 0.00 | 4.00 ± 0.01 | 4.00 ± 0.00 | 3.90 ± 0.01 | 3.87 ± 0.01 |
| Control without preservatives | 4.00 ± 0.01 | 3.92 ± 0.01 | 3.81 ± 0.01 | 3.74 ± 0.01 | 3.60 ± 0.01 | 3.45 ± 0.00 |

**Effect of different concentrations of EOs in the absence of synthetic preservatives on organoleptic properties of labneh**

The organoleptic properties of the different labneh samples were also investigated and the results were presented in Table 7. The total scores of labneh containing EOs decreased with an increase in the concentration of the EOs. In addition, in all cases the total scores of the sensory evaluation decreased gradually during storage. The best oil and most acceptable oil were rosemary at followed by sweet almond. It is noted that the sweet almond oil does not have the strong taste or distinctive taste, but the evaluation was not very good, especially in the last weeks because the taste of acidity in labneh sample.

**Table 7.** Organoleptic properties of labneh treated with sweet almond, clove, eucalyptus, rosemary EOs for 6 weeks

| Oil | Concentration(ppm) | Fresh labneh | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 |
|---|---|---|---|---|---|---|---|---|
| **Positive Control** | 300 | 96 | 96 | 93 | 91 | 87 | 82 | 77 |
| **Negative Control** | 0 | 96 | 93 | 86 | 82 | 71 | 66 | 59 |
| **Sweet almond** | 300 | 96 | 92 | 90 | 83 | 76 | 77 | 72 |
| **Sweet almond** | 400 | 96 | 90 | 91 | 82 | 79 | 75 | 73 |
| **Sweet almond** | 500 | 96 | 89 | 90 | 87 | 83 | 80 | 75 |
| **Sweet almond** | 600 | 96 | 87 | 86 | 83 | 76 | 73 | 70 |
| **Clove** | 300 | 96 | 80 | 82 | 77 | 75 | 71 | 68 |
| **Clove** | 400 | 96 | 73 | 70 | 68 | 63 | 58 | 52 |
| **Clove** | 500 | 96 | 70 | 67 | 67 | 60 | 61 | 54 |
| **Clove** | 600 | 96 | 66 | 62 | 58 | 52 | 50 | 50 |
| **Rosemary** | 300 | 96 | 90 | 88 | 86 | 82 | 80 | 78 |
| **Rosemary** | 400 | 96 | 91 | 91 | 91 | 84 | 81 | 75 |
| **Rosemary** | 500 | 96 | 88 | 90 | 84 | 80 | 76 | 70 |
| **Rosemary** | 600 | 96 | 86 | 83 | 79 | 80 | 75 | 73 |

## CONCLUSION

EOs have a wide spectrum of antimicrobial activity, their use as preservatives in food has not yet been extended. In the last few decades, consumers are demanding healthy safe food with least concentration of synthetic food additives and least heat treatment. EOs represent an alternative to synthetic preservatives in the food industry against spoilage bacteria especially *coliforms*, *E. coli O157:H7*, yeast, mold, *S. aureus* which were tested in this study. Most of the selected plant extracts used in this study, have antimicrobial active compounds of that could substitute natamycin, sodium benzoate and potassium sorbate.

Labneh is a middle eastern fermented milk, that is highly consumed but with a major problem in its short shelf life due to contamination during processing, leading to use of synthetic potassium sorbate at different concentrations. The addition of EOs can be used as a single substitute to potassium sorbate to increase the shelf life, or by the combination of natural preservatives and synthetic preservatives leading to better results using low concentration of synthetic antimicrobial agents (150ppm of potassium sorbate). According to our study, there are two possibilities either using natural plant extracts as substitutes and /or use in combination with synthetic antimicrobial agent. Our results showed that clove and rosemary essential oil at 600 µl/kg can be used to increase the shelf life of labneh for up to 6 weeks at 5 ± 1ºC with acceptable taste, flavor, and texture.

Rosemary and clove EOs at concentrations of, 600 µl/kg can be used to increase the shelf life of labneh for up to 6 weeks without any synthetic preservatives. An increase in the EOs concentrations leads to a decrease in bacterial, yeast and mold counts. Both coliform and E. coli were not detected in any of the labneh samples prepared by addition of the respective EOs. The choice of an essential oil and its concentration in a particular food is important, because a small amount can cause sensory alterations. Clove oil and rosemary oil have good antiseptic, antibacterial

and antifungal properties compared to other oil used in this study, because of the presence of phenols, monoterpene, alcohols, aldehydes esters and lactones which affect the growth of pathogenic microorganisms especially gram positive. Although the literature data about the antimicrobial effect of EOs are abundant, there are new areas of application to be discovered especially the effect of the chemical composition and its physicochemical effects. Extraction of the active ingredients of these oils or other oils and their applications as preservatives or antioxidants on food may give appreciable results.

## REFERENCES

**[1]** Davidson, P.M. and Taylor, M.T. (2007) Chemical Preservatives and Natural Antimicrobial Compounds, Food Microbiology, 713-745.

**[2]** Farkas, J. (2007). "Physical methods of food preservation", in food Food Microbiology: Fundamentals and Frontiers, eds. P. Doyle, L. R. Beuchat, and T. J. Montville (Washington, DC Society for Microbiology Press), 685–705.

**[3]** Arques, J., Rodriguez, E., Nunez, M., & Medina, M. (2008). Inactivation of gram negative pathogens in refrigerated milk by reuterin in combination with nisin or the lactoperoxidase system. European Food Research and Technology, 227(1), 77–82.

**[4]** Aslim, B., & Yucel, N. (2007). In vitro antimicrobial activity of essential oil from endemic Origanum minutiflorum on ciprofloxacin-resistant *Campylobacter* spp. Food Chemistry, 107(2), 602–606.

**[5]** Appendini. P, Hotchkiss, J. (2002). Antimicrobial food packaging - a review. Food Science & Emerging Technologies 3(2):113-126 ·

**[6]** Hanušová, K, Dobiáš J., Klaudisová K. (2009). Effect of packaging films releasing antimikrobial agents on stabil-ity of food products. Czech Journal of Food Sciences, 27 (Special Issue): S437–S439.

**[7]** Burt, S. (2004). EOs: their antibacterial properties andpotential applications in foods—a review. International Journal of Food Microbiology 94 (2004) 223–253.

**[8]** Ponce, A.; Del Valle, C.; Roura, S (2004). Shelf life of leafy vegetables treated with natural essential oils. J. Food Sci. 69, 50–56.

**[9]** Gutierrez, J., Rodriguez, G., Barryryan, C., Bourke, P. (2008). Efficacy of plant EOs against food borne pathogens and spoilage bacteria associated with ready-to-eat vegetables: Antimicrobial and sensory screening. Journal of Food Protection, 71(9), 1846–1854. (B).

**[10]** Gutierrez, J., Barryryan, C., Bourke, P. (2009). Antimicrobial activity of plant EOs using food model media: Efficacy, synergistic potential and interactions with food components. Food Microbiology, 26, 142–150.

**[11]** Dorman, H., Deans, S., (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. Journal of Applied Microbiology 88, 308– 316.

**[12]** Skandamis, P., Nychas, G. (2000). Development and evaluation of a model predicting the survival of Escherichia coli O157:H7 NCTC 12900 in homemade eggplant salad at various temperatures, pHs and oregano essential oil concentrations. Applied and Environmental Microbiology 66 (4), 1646–1653.

**[13]** Mangena, T, Muyima, N. (1999). Comparative evaluation of the antimicrobial activities of EOs of *Artemisia afra, Pteronia incana* and *Rosmarinus officinalis* on selected bacteria and yeast strains.

**[14]** Marino, M., Bersani, C., Comi, G., 2001. Impedance measurements to study the antimicrobial activity of EOs from *Lamiacea* and *Compositae*. International Journal of Food Microbiology 67, 187– 195.

**[15]** Muir.,D, Banks., J. (2000). The Stability and Shelf Life of Food. Wood head Publishing Limited.

**[16]** Draughon, F.A. (2004). Use of Botanicals as Biopreservatives in Foods. Food technology, 58(2): 20-28.Obst, J.R.

**[17]** Thabet, H., Nogaim Q., Qasha A., Abdoalaziz O., Alnsheme N. (2014). Evaluation of the effects of some plant derived EOs on shelf life extension of Labneh. Merit Research Journal of Food Science and Technology (ISSN: 2354-2527) Vol. 2(1) pp. 008-014.

**[18]** Robinson RK, Tamime AY (1994). Manufacture of yogurt and other fermented milks. In Robinson RK (Ed.). Modern dairy technology, Advances in milk products.. London: Elsevier Applied Science. 2: 1- 48.

**[19]** Tamime A.Y. and Robinson R.K., (2007) Tamime and Robinson's yoghurt science and technology, third edition. Woodhead Publishing Limited, England.

**[20]** Hüsnü, K., Gerhard B. (2010). EOs Science, Technology, and Applications, Taylor and Francis Group, LLC, New York.

**[21]** Carson, C. F., & Hammer, K. A. (2011). Chemistry and bioactivity of essential oils. Lipids Essent Oils Antimicrob Agents, *25*, 203-38.

**[22]** Fisher, K., & Phillips, C. A. (2006). The effect of lemon, orange and bergamot essential oils and their components on the survival of Campylobacter jejuni, Escherichia coli O157, Listeria monocytogenes, Bacillus cereus and Staphylococcus aureus in vitro and in food systems. Journal of applied microbiology, *101*(6), 1232-1240.

**[23]** Bajpai, V., Rahman, A., Kang, S. (2008). Chemical composition and inhibitory parameters of essential oil and extracts of Nandina domestica Thunb to control food-borne pathogenic and spoilage bacteria. International Journal of Food Microbiology, 125(2), 117–22.

**[24]** Ceylan, E., Fung, D. (2004). Antimicrobial activity of spices. Journal of Rapid Methods and Automation in Microbiology, 12(1), 1–55.

**[25]** Angioni, A., Barra, A., Cereti, E., Barile, D., Coisson, D. J., Arlorio, M., et al. (2004). Chemical composition, plant genetic differences, antimicrobial and antifungal activity investigation of the essential oil of *Rosmarinus officinalis* L. Journal of Agricultural and Food Chemistry, 52(11), 3530–3535.

**[26]** Daferera, D., Ziogas, B., Polissiou, M. (2000). GC-MS analysis of EOs from some Greek aromatic plants and their fungitoxicity on *Penicillium digitatum*. Journal of Agricultural and Food Chemistry 48, 2576–2581.

**[27]** Davidson, P., Naidu, A. (2000). Phytophenols and Natural food antimicrobial systems. 265–295, Boca Raton, Florida: CRC Press.

**[28]** Delaquis, P., Stanich, K., Girard, B., Mazza, G. (2002). Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus EOs.

**[29]** Ling ER (1963). A Text Book of Dairy Chemistry. 2: 3 Ed. Chapman and Hall Ltd, London. pp. 63-79.

**[30]** Klose J (1968). Harmonisierung des Speiseeisrerchtes in der EWG. Subwaren. 14: 778 -780.

**[31]** Harrigan, W.F and McCance, E.M. (1966) Laboratory Methods in Microbiology. Vol. 54, Academic Press, Cambridge, 970.

**[32]** American Public Health Association (1978). Standard Method for the Examination of Dairy Products. 14 Ed. Washington, USA.

**[33]** Keating K, Randwhite S (1990). Effect of alternative sweeteners in plain and fruit flavored yoghurt. J. Dairy Sci. 37-54.

**[34]** Laekeman G. M., Van Hoof L., Haemers A., Berghe D. A. V., Herman A. G., Vlietinck A. J. (1990). Eugenol a valuable compound for in vitro experimental research and worthwhile for further in vivo investigation. Phytother. Res. 4, 90–9610.1002/ptr.2650040304

**[35]** Pauli A., Kubeczka K. H. (2010). Antimicrobial properties of volatile phenylpropanes. Nat. Prod. Commun. 5, 1387–1394

**[36]** Gill, A., Holley, R. (2006). Disruption of *Escherichia coli*, *Listeria monocytogenes* and *Lactobacillus sakei* cellular membranes by plant oil aromatics. *Int.J. Food Micro.*108, 1–9. (A).

**[37]** Hemaiswarya S., Doble M. (2009). Synergistic interaction of eugenol with antibiotics against Gram negative bacteria. Phytomedicine 16, 997–100510.1016/j.phymed.2009.04.006

**[38]** Dipasqua, R., Hoskins, N., Betts, G., Mauriello, G. (2006). Changes in membrane fatty acids composition of microbial cells induced by addiction of thymol, carvacrol, limonene, cinnamaldehyde, and eugenol in the growing media. J. Agric. Food Chem. 54, 2745–2749.

**[39]** Dipasqua, R., Betts, G., Hoskins, N., Edwards, M., Ercolini, D., Mauriello, G. (2007). Membrane toxicity of antimicrobial compounds from EOs. J.Agric. Food Chem. 55, 4863–4870.

**[40]** Bennis, S., Chami, F., Chami, N., Bouchikhi, T., Remmal, A. (2004). Surface alteration of *Saccharomyces cerevisiae* induced by thymol and eugenol. Lett. Appl. Microbiol. 38, 454–458.

**[41]** Ismail AM, Harby S, Salem AS (2006). Production of flavored labneh with extended shelf life. Egyptian J. Dairy Sci. 34: 59-68.

**[42]** Tamime AY (1978a). Concentrated yoghurt "Labneh" a potential new dairy spread. The Milk Industry. 80(3): 4-7.

**[43]** Tamime AY (1978b). The production of yoghurt and concentrated yoghurt from hydrolyzed milk. Cult. Dairy. Prod. J. 13(3): 13-16.

**[44]** Tamime AY, Robinson RK (1985). Yoghurt Sciences and Technology. I, T Ed.Weaton and Co. Ltd., England. pp. 209-213

**[45]** Abbas., F, Osman., M. (1998). Properties of labneh like products manufactured using acid and acid rennet coagulation. Ann. Agric. Sci. Moshtohor., 36(1): 401-411.

**[46]** Rosenthal et al., 1980; Guler, 2007; Abou Ayana and Gamal El Deen, 2011 and Senel et al., 2011).

**[47]** Guler, 2007; Changes in salted yoghurt during storage, International Journal of Food Science & Technology 42(2):235 - 245

**[48]** Abou Ayana and Gamal El Deen, 2011; Improvement of the Properties of Goat's Milk Labneh using some Aromatic and Vegetable Oils International Journal of Dairy Science 6(2):112-123

**[49]** Senel E, Atamer M, Gursoy A, FS Oztekin (2011). Changes in some properties of strained (Suzme) goat's yoghurt during storage. Small Rumin.Res., vol. 99, pp. 171–177.

# ALTERNATIVE EXTRACTION TECHNIQUES OF CURCUMINOIDS FROM TURMERIC*

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## ABSTRACT

Curcumin, demethoxycurcumin, and bisdemethoxycurcumin have recently been the focus of attention on food science due to their growing popularity among health-conscious consumers. Traditionally, curcumin has been used as a colorant, a sweetener, and a food preservative. Natural plants contain various bioactive components such as lipids, phytochemicals, compounds used in pharmacology, flavors, odors, and pigments, so extracts of these plants are often used in industries such as pharmaceuticals, food, and cosmetics. Some traditional and mechanical processes are used to achieve maximum benefit in the commercial use of these high-cost compounds. Alternative techniques are used to overcome the disadvantages of traditional extraction methods. These techniques have been developed to overcome these disadvantages and, most importantly, maintain the

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integrity of the compounds and achieve an environmentally friendly process Developed as an alternative to traditional methods to extract chemicals from plant sources, ultrasound-assisted extraction (UAE), accelerated solvent extraction (ASE), supercritical fluid extraction (SFE), subcritical water extraction (SWE), microwave assisted extraction and enzyme-assisted extraction (EAE) methods, such as fast, effective, and relatively environmentally friendly compared to the organic solvents used are considered.

**Keywords:** Curcumin, *Curcuma longa* L., Extraction, Alternative techniques, Green extraction

## INTRODUCTION

Turmeric (*Curcuma longa* L.) belongs to the genus *Curcuma*, which is in the family *Zingiberaceae*, where ginger and cardamom are also present, and consists of hundreds of plant species **[1].** Turmeric is widely grown in countries and regions with tropical and subtropical climates, especially in China, India, and Indonesia, as well as in some Latin American countries such as Brazil and Peru **[2].**

A group of phenolic components responsible for the yellow color in the roots of turmeric was isolated in the 19th century and named after curcumin. Curcuminoids found in turmeric, the main component of which is curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], consist of three main active components: curcumin, demethoxycurcumin and bis-demethoxycurcumin (Figure 1.) **[1, 3, 4].** Commercially available curcumin contains 77% of curcumin as well as other curcuminoids. Commercial curcumin contains about 75% of the total curcuminoids, while demethoxycurcumin contains 10-20% and bisdemethoxycurcumin usually contains <5% **[5].** These compounds are included in the group called diarileptanoids **[6].**

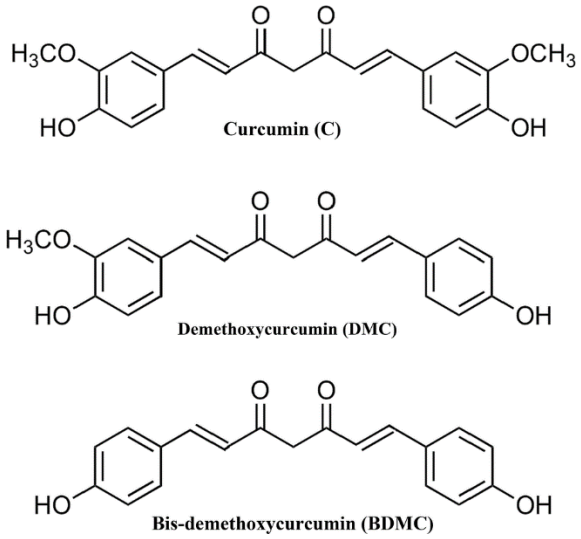


**Figure 1**. Chemical structure of curcuminoids **[7].**

Curcumin is the most active ingredient in turmeric, making up 2-5% of turmeric and is a water-insoluble compound. It was first isolated from curcuminoids in the form of a yellow-orange crystalline powder by Vogel in 1815 **[8].** The first chemical formula of curcumin ($C_{21}H_{20}O_6$) was described as diferulomethane by Lampe and Milobedeska in 1910 **[9].** The yellow color of turmeric's rhizomes is due to the presence of a group of phenolic compounds called curcuminoids **[2].** Curcumin has two methoxy groups and has a reddish orange color; demethoxycurcumin has a single methoxy group and has an orange-yellow color, and bisdemethoxycurcumin can be distinguished by its yellow color, while it does not contain methoxy groups **[1, 10].**


**Figure 2.** Representation of curcuminoids health benefits **[11].**

**Importance of curcumin**

Curcumin is a natural food dye of yellow-orange color that has the code E100 according to the European Food Safety Authority food dye numbering **[3].** In addition, it is also widely used as a preservative and aroma/flavoring agent **[2].** Curcuminoids belonging to the diferuloylmethane group have been used in Asia for centuries as spices, natural colorants, and traditional medical materials **[12].** The acceptable daily intake limit of curcumin has been determined by the World Health Organization (WHO) as 0-3 mg/kg. Products containing curcuminoids and turmeric have been described as safe by the Food and Drug Administration (FDA) in the United States **[11].**

Due to its wide range of biological activity, turmeric, and the curcumin, have been the subject of many studies. Curcuminoids have been used to treat a wide range of diseases such as cancer, inflammation, hepatic diseases, and diabetes (Figure 3) **[2, 13, 14, 15].** In 1949 they were discovered to have antibacterial properties **[16].**

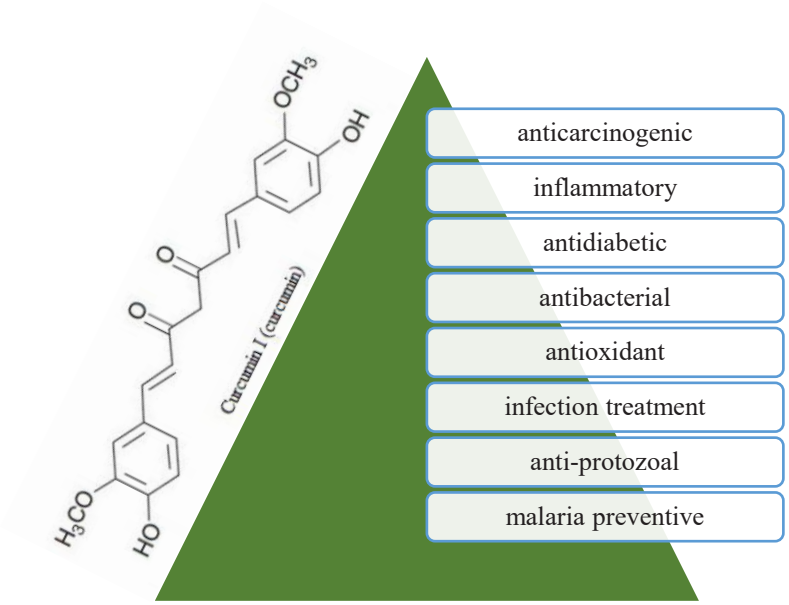


**Figure 3.** Chemical structure of curcumin and health benefits of curcuminoids

These compounds also have free radical cleansing antioxidant properties **[17, 18, 19]** are inhibitors of the human immunodeficiency virus Type 1 (HIV-1) integrase and are used to treat infection **[20, 21].** Many studies have shown that demethoxycurcumin and bisdemethoxycurcumin have as strong biological activity as curcumins in terms of anti-inflammatory and anti-protozoal properties **[22, 23, 24].** In addition, it has been noted in some studies that these compounds exhibit anti-malaria activity in vitro and in vivo **[25].** Although it is known that 3 compounds have a different effect, it has been observed that mixtures of these compounds prepared in certain proportions have more than the effect created by a single compound, creating a synergistic effect **[26].**

## Extraction

Traditional extraction techniques such as traditional solid-liquid extraction, sonication, soxhlet extraction have been used to extract curcuminoids **[27].**

Curcuminoids are sensitive to light in the solution state and in the solid state, and if the pH of the solution is high, they undergo hydrolytic degradation **[28].** As a result of the application of traditional extraction methods of these active compounds, degradation of the extracted curcuminoids occurs due to their exposure to light, oxygen, and high temperatures. Because of this, extraction efficiency and application of the obtained compounds in food products may become limited **[29].** As a result of traditional methods used in mechanical processes, low extraction efficiency is achieved, as well as organic solvents that are harmful to the environment and human beings are used in traditional extraction methods **[30, 31].** Furthermore, because many natural products are thermally unstable during thermal extraction, degradation can be experienced **[30, 31, 32, 33].** Based on literature, traditional techniques usually have low extraction efficiency, high temperature processes, and longer extraction times **[34, 35].** The biggest disadvantage of traditional extraction methods is high energy consumption **[36].**

As a result of traditional techniques, low extraction efficiency is encountered despite high operating costs. Alternative techniques have been developed to overcome these disadvantages and, most importantly, maintain the integrity of the compounds and achieve an environmentally friendly process. Strict guidelines reported by the authorities on the use of organic solvents have encouraged researchers to develop cleaner/environmentally friendly extraction technologies **[37].** Demand for new extraction techniques is increasing due to short extraction time, low consumption of organic solvents and the desire to avoid increased pollution **[38].** Developed as an alternative to traditional methods to extract chemicals from plant sources, ultrasound-assisted extraction (UAE), accelerated solvent extraction (ASE), supercritical fluid extraction (SFE), subcritical water extraction (SWE), microwave assisted extraction (MAE) and enzyme-assisted extraction (EAE) methods, such as fast, effective, and relatively environmentally

friendly compared to the organic solvents used are considered. Alternative extraction techniques of curcuminoids from turmeric is shown in Table 1.

**Ultrasound-Assisted Extraction**

Ultrasonic-assisted extraction (UAE) as a new technique for the extraction of plant tissues has received increasing attention and has also been the subject of many studies **[49, 50, 51].** Ultrasound has been noted to be effective in increasing the extraction rate by increasing mass transfer rates and cell wall breakdown due to the formation of microcavities leading to higher product yields with shorter extraction time and less solvent consumption **[52].** In other words, combining solvent extraction applied to the material with ultrasound has been reported to increase mass transfer and solvent penetration into the plant material by destroying the cell walls due to the mechanical effects of acoustic cavitations **[31, 35].** It is essential to optimize ultrasound extraction system parameters such as solvent, polarity, duration, pH to increase high extraction efficiency when obtaining desired compounds from plant materials **[5].**

A study by Rouhani et al. **[5]** compared traditional methods with ultrasound-assisted extraction of curcuminoids from turmeric samples. Extraction was performed in ultrasonic bath at 35 KHz and 25 °C and optimization was performed with independent variables. 3 different levels of 3 different factors were used in the optimization extraction process (pH-3/6/9; ethanol - 70/80/90%;

**Table 1.** Alternative extraction techniques of curcuminoids from turmeric

| Method | Material | Extraction Medium | Reference |
|---|---|---|---|
| UAE, CE, SE | *Curcuma longa* L. | Ethanol | **[5]** |
| UAE, CE, SE | *Curcuma amada* | Acetone, ethanol, methanol, ethyl acetate and water | **[36]** |
| PLE, UAE | *Curcuma longa* L. | Methanol | **[28]** |
| PLE, UAE, MAE, SE | *Curcuma wenyujin Y.H.chen et C.Ling* | Methanol | **[39]** |
| SCFE, UAE, MAE, SE | *Curcuma longa* L. | $CO_2$, ethanol, acetone, water, | **[40]** |
| SCFE | *Curcuma longa* L. | Millii-Q water | **[41]** |
| USC-$CO_2$E, SC-$CO_2$E | *Curcuma longa* L. | $CO_2$, ethanol | **[42]** |
| SWE, SE | *Curcuma longa* L. | Acetone, ethanol, isopropanol, water | **[3]** |
| SWE | *Curcuma longa* L. | Water | **[43]** |
| MAE | *Curcuma longa* L. | Ethanol | **[44]** |
| MAE, CE | *Curcuma longa* L. | Ethanol, methanol | **[45]** |
| MAE | *Curcuma longa* L. | Acetone, acetic acid, ethanol, methanol, water | **[46]** |
| UAE, MAE, EAE, CE | *Curcuma longa* L. | Acetone, α-amylase, amyloglocosidase | **[47]** |
| CLAE, EAE, CE | *Curcuma longa* L. | *Acetone*, α-amylase, amyloglocosidase, *N*, *N*-Dipropyl ammonium *N*′, *N*′-dipropylcarbamate (DPCARB) | **[48]** |

Ultrasound-assissted extraction (UAE), Conventional extraction (CE), Soxhelet extraction (SE), Pressurized liquid extraction (PLE), Supercritical fluid extraction (SCFE), Supercritical carbon dioxide extraction (SC-$CO_2$E), Ultrasound-assisted supercritical carbon dioxide extraction (USC-$CO_2$E), Subcritical water extraction (SWE), Microwave-assisted extraction (MAE), Enzyme-assisted extraction (EAE), Carbamate ionic liquid assisted extraction (CLAE)

time-5/10/15min). The optimal combination of the factors of the parameters was determined as ethanol/water 70:30 v/v, pH 3 and 15 minutes. The ultrasound-assisted extraction yield was found to be about 3 times higher compared to the traditional method.

A study by Shirsath et al. **[36]** compared ultrasound-assisted extraction of curcumin from *Curcuma amada* (mango ginger) with traditional method extraction. In extraction, the effect of different parameters such as solvent (ethanol, methanol, acetone, ethyl acetate and water), solvent/solid ratio (1:15-1:55), particle size (0.09-0.85 mm), temperature (25- 55°C) and ultrasound power (22 kHz frequency 130-250 W) on extraction efficiency was studied. As a result of the study, ethanol was selected as the best extraction solvent. Curcumin extraction yield; increased with the increase of temperature, increased with increase of input power and increased with the decrease of particle size. According to the data obtained, curcumin extraction was performed optimally with 72% efficiency (9.18 mg/g) at 35°C, 1 hour, 1:25 solid/solvent ratio, 0.09 mm particle size and 250 W ultrasound power at 22 kHz frequency. The most important benefit of ultrasound-assisted extraction has been seen to shorten the extraction time. It has been stated that the extraction of thermally unstable components from plant materials can be carried out at low temperatures and the degradation of the components can be prevented.

**Accelerated Solvent Extraction**

Pressurized liquid extraction (PLE) or accelerated solvent extraction (ASE), as we can see in other sources, is a more attractive alternative than traditional extraction methods due to the need for fewer solvents and being more efficient **[53].** Today, it is widely known that PLE is a common green approach for the extraction of target compounds found in plant plants **[54].** PLE overcomes the

disadvantages of traditional extraction methods and has often been used for analytical purposes in the preparation of samples. In addition to being a technique that can be easily automated and characterized, the main reason it is used as an alternative method is that it has a low cost and positive environmental impact due to low solvent use **[55].** PLE can be used over a wide temperature range (313-473 K) and at medium to high pressures (3.5-35 MPa) (Figure 4.). The main reason it is used in these values is to shorten the extraction time and keep the solvent in the compressed liquid region **[56].** The most important features that increase the extraction efficiency in this method are increased temperatures, increased mass transfer rate and diffusion rates **[53].**

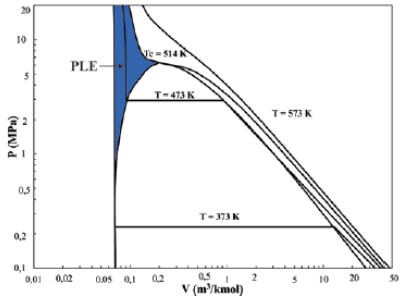


**Figure 4.** Pressure-volume diagram for ethanol calculated using Peng-Robinson equation **[57].**

A study by Schieffer **[28]** compared the extraction of curcuminoids from Curcuma longa with pressurized liquid extraction and ultrasound-assisted extraction. At a temperature of 373 K, under a pressure of 10 MPa, statically pressurized liquid extraction was performed for 5 minutes using methanol as a solvent. According to the study, higher performance was observed in pressurized liquid extraction in terms of curcuminoid extraction.

In a study by Shen et al. **[39]**, pressurized liquid extraction of curcuminoids from *Curcuma wenyujin Y.H.chen et C.Ling* (*Curcuma aromatica* Salisb.) using

methanol as a solvent at a temperature of 100 °C under a pressure of 1500 psi was carried out. Compared to other extraction methods (soxhelet, ultrasound-assisted, microwave-assisted extraction), it was observed that it required a shorter extraction time and less solvents.

**Supercritical Fluid Extraction**

Another method that can be described as an alternative is supercritical fluid extraction (SFE). In SFE, the solvent used in extraction is in a critical state. A substance or mixture that is pressurized above the critical pressure and heated above the critical temperature that is unique to the fluid is called a supercritical fluid (Figure 5). Supercritical fluids carry the properties of the intermediate form of deciduous gas or liquid matter. They cannot be liquefied or evaporated by increasing pressure or temperature, so they exist in a single phase. Its most important features are higher diffusion coefficients and lower viscosity. Its dissolving and spreading properties are higher than those of liquids, and its reaction kinetics are fast. Because of these properties, supercritical fluids have a high ability to penetrate solid porous materials **[58, 59].** The higher the density of supercritical fluids, the higher the ability to dissolve. Because density and other properties can be easily changed by adjusting temperature and pressure, these fluids are seen as ideal solvents **[60].**

Carbon dioxide ($CO_2$) is the most used and preferred supercritical solvent in food applications due to its cheap, high purity, easy availability, reliability, and many properties **[61].** Supercritical $CO_2$ (SC-$CO_2$) is slightly polar or non-polar compounds can solve; low-molecular-weight compounds can solve; for it is a low resolution free fatty acids and glycerides; high molecular weight and/or pressure with an increase in more polar compounds can separate; solvent property for pigments is low, it cannot dissolve proteins, polysaccharides, sugars, and mineral salts **[62, 63].**

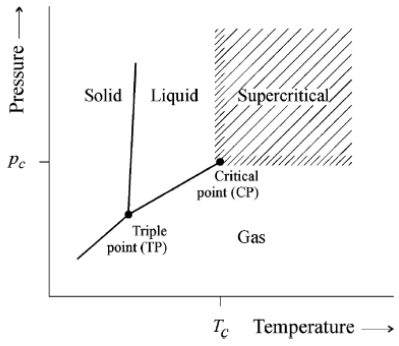


**Figure 5.** Diagram of supercritical state for a pure component **[65].**

In a study conducted by Kimthet et al. **[42]**, ultrasound-assisted supercritical carbon dioxide extraction (USC-$CO_2$) method was used to obtain curcumin from *Curcuma longa* L. Extraction was carried out at 50°C, under pressure of 25 MPa, $CO_2$ as solvent and 10% ethanol (flow rate 3 mL/min) as cosolvent. As a result of the study, it was determined that USC-$CO_2$ requires less extraction time compared to SC-$CO_2$ and a higher percentage of curcumin is obtained as a result of extraction. It has been reported that the reason for this is that ultrasound power breaks down the cell wall and therefore increases the release of target components.

In a study conducted by Kwon & Chung **[41]**, subcritic solvent extraction of curcuminoids in turmeric was performed. Different parameters (temperature as 110-150 °C; time as 1-10 min; pressure as 5-100 atm; solid-to-solvent ratio and mixing ratio of solvent) were examined in the study. The maximum extraction yield of the study was 13.58% (curcumin, demethoxycurcumin and bisdemethoxycurcumin; 4.94%, 4.73% and 3.91% in dried extracts, respectfully) in pilot-scale assembly (water/ethanol mixture at 135 °C in 5 minutes and solvent (50:50, v/v)). When using ethanol (50%, 95% and 100%) in different proportions for 120 minutes at 60 °C under atmospheric conditions, extraction yields of

10.49%, 13.71% and 13.96% were obtained, respectively. According to the data obtained from the study, supercritical solvent extraction has been suggested as a potential alternative to the extraction of curcuminoids as a fast and efficient extraction technique.

**Subcritical Water Extraction**

Subcritical Water Extraction (SWE); also known as Pressurized hot water extraction (PHWE), hot water extraction (HWE), high-temperature water extraction (HTWE), superheated water extraction or hot liquid water extraction, due to the use of organic solvents, is considered to be an environmentally friendly extraction method that can be a potential alternative to the traditional extraction method **[3, 64].** The use of pure water as a solvent at high temperatures for nonpolar analytes was first proposed by Hawthorne **[65].** This extraction method has been put forward as a promising green technique, based solely on the use of subcritical water solvent **[66, 67, 68].** The term "pressurized hot water" or "subcritical water" usually describes liquid water with the temperature of 647.096 K (374 °C) and the pressure of critical point 22.064 MPa **[3].** It has been suggested that subcritical water is more effective than SC-$CO_2$ for modifying sample matrices, rearranging analyte binding sites, and organic matter extraction **[69].**

A study by Euterpio et al. **[3]** shows that curcumin increases temperature to improve pressurized hot water extraction due to its low solubility in water used as a solvent. In the experiment, it was observed that at temperatures above 473 K, both the turmeric matrix and curcumin deteriorated, as well as the color of the extracted ginger rhizome particles turned dark brown. In the dynamic extraction process with a solvent speed of 0.5 mL/min under a pressure of 5 MPa at 370 K, the solubility of curcumin was increased by improving the solvent water with a buffer solution with a pH of 1.6 and using a phosphate buffer of 62 g/L.

In a study by Kiamahalleh et al. **[43],** subcritical water extraction of curcumin from turmeric root with different parameters such as pressure and time on the extraction efficiency was investigated. Optimum conditions are achieved with a temperature of 150 ◦C, a pressure of 10 bar, a particle size of 0.71 mm and time of 14 min, and the maximum extraction efficiency obtained under these conditions is 3.8%.

**Microwave-Assisted Extraction**

Microwave systems are systems where heat is generated through interaction between ions as well as the dipole rotation resulting from the high-frequency electromagnetic waves **[60].** They possess significant advantages over other common methodologies such as performing the extraction with high efficiency, making it possible to use less solvent and reducing the extraction time by some margin **[70].** Extraction is characterized by microwave radiation penetrating the cell walls and membranes, breaking down the cell structure, which makes it easier for solvent to permeate into the cells **[71].** It is also possible to influence the permeating capability of the solvent by changing its temperature **[70].** With the effect of the electric field created by the microwave energy, the transmission of electrons in the material structure is disrupted and the dipoles rotate around their own axis **[72].** The greater the resistance of electrons to motion and the continuous change of direction of ions or the dipole oscillation of the molecule, the higher the heat generated **[72, 73].**

The heating profiles of the materials depend on their dielectric properties, mainly dielectric constants, and dielectric losses **[74].** The resistance of the material against the passage of microwave energy is defined as the dielectric constant, and this energy turning into heat and dissipating in the material is defined as dielectric loss. Differences in dielectric properties of solvents such as ethanol, methanol,

acetone, and water, which are frequently used in studies where microwave-assisted curcuminoid extraction is performed, affect the extraction properties of curcuminoids substantially **[44, 45, 46, 47, 75].** For example, while ethanol has lower dielectric constant than water, its dielectric loss is higher **[76].** In this case, ethanol is less resistant to the passage of microwave energy, while its ability to convert this energy into heat is higher and needs less energy, i.e., shorter times, to reach a certain temperature.

The most frequently used solvents are ethanol and acetone, which are not remarkably similar in terms of dielectric characteristics **[77].** While the dielectric constants of these two solvents are similar, it has been observed that the dielectric loss of ethanol is much higher **[76].**

Rezaei et al. **[46]** examined different solvents for curcumin extraction in their study and stated that acetone is the solvent that provides the extraction with the highest efficiency. A relationship could not be established between the dielectric constant of the solvents and the amount of extractable curcumin; however, it was stated that there was a direct relationship with the deterioration of the cell structure. This also makes the use of microwave systems for pre-application in curcumin extraction a possible approach.

Wakte et al. **[40]** investigated microwave application to ginger powders soaked with ethanol or water as a pre-treatment. It was observed that the extraction efficiency of the samples that were applied microwave pre-treatment with water with higher dielectric constant than ethanol was higher. In addition, microwave pre-treatment was found to be much more efficient at breaking down cell structure than ultrasound-assisted approach.

Sahne et al. **[47]** examined the extraction of curcumin from ginger using enzyme, ultrasound and microwave-assisted methods. Although the extraction efficiency

was 3.72% in microwave-assisted extraction and 4.1% in enzyme-assisted extraction, extraction times of 2 minutes and 4 hours, respectively, created a significant advantage for microwave-assisted extraction.

**Enzyme-Assisted Extraction**

Nowadays, with the increase of consumer awareness, interest in organically produced foods has increased, while recent studies have focused on examining green alternatives to existing extraction techniques **[78].** Therefore, enzyme-assisted extraction (EAE), which is known to be organic and environmentally friendly, has been one of the most popular methods and numerous studies have shown that enzyme-assisted applications have high efficiency in the extraction of bioactive components such as lipids, polyphenols, oils, and aroma elements **[79, 80, 81, 82].** It is important to determine an enzyme suitable for the subject food, since the enzymes used show significant differences in extraction efficiency depending on the activity, the amount of substrate and the molecular composition of bioactive components **[80].** α-amylase, glucoamylase and amyloglucosidase were generally used in studies due to approximately 65% of curcumin consisting of carbohydrates and curcumin residing in the polysaccharide-lignin structure **[47, 48, 83, 84].** With enzymatic applications, the structure of the ginger cell wall is disrupted, and the solvent transition is facilitated, thus ensuring high efficiency extraction without the need for very high temperatures **[83].**

Kurmudle et al. **[84]** showed that the α-amylase and glucoamylase-assisted extraction system was 26.04% and 31.83% more efficient, respectively, compared to the system without enzyme assistance.

Sahne et al. **[47]** have compared ultrasound, microwave, and enzyme-assisted extraction of bioactive compounds in ginger and determined that enzyme-assisted extraction was performed with the highest efficiency among the three methods

examined. It has been reported that with enzyme-assisted application, cell walls can be broken down more effectively and enable solvent to permeate far more effectively.

## CONCLUSION

Curcumin, which has many positive health effects, is used as a natural color pigment, and is usually derived from turmeric, has an important place in the industrial sense as a multidisciplinary. Traditional methods for the extraction of curcumin from turmeric are still used today. But extraction methods such as UAE, ASE, SFE, SWE, MAE and EAE, which can be described as alternative methods, are known to overcome the disadvantages of traditional methods, achieving better extraction yields, and using solvents that are less harmful to nature. Each Extraction method has advantages and disadvantages compared to each other. Alternative and environmentally friendly extraction methods can be used for recovery instead of conventional (traditional) methods that can be applied in every laboratory with a low budget but require low extraction efficiency and high solvent use. After selecting the method, parameters such as the appropriate solvent type, solvent quantity and extraction temperature should be considered, and extraction should be supported by experimental design and optimization for maximum extraction efficiency and optimal conditions should be determined. Of course, determining the most appropriate extraction method in which maximum efficiency is achieved by using environmentally friendly and low-cost solvents at temperatures that will prevent curcumin from becoming degraded should be the first step. Developing technology will bring with it different alternative techniques. The goal of curcumin extraction techniques to be developed should be to require environmentally friendly solvent use (or reduction of solvent use), high extraction efficiency, low process costs and a short extraction time.

## REFERENCES

**[1]** Jayaprakasha, G. K., Rao, L. J. M., & Sakariah, K. K. (2005). Chemistry and biological activities of *C. longa*. Trends in Food Science & Technology, 16(12), 533-548.

**[2]** Hatcher, H., Planalp, R., Cho, J., Torti, F. M., & Torti, S. V. (2008). Curcumin: from ancient medicine to current clinical trials. Cellular and molecular life sciences, 65(11), 1631-1652.

**[3]** Euterpio, M. A., Cavaliere, C., Capriotti, A. L., & Crescenzi, C. (2011). Extending the applicability of pressurized hot water extraction to compounds exhibiting limited water solubility by pH control: curcumin from the turmeric rhizome. Analytical and bioanalytical chemistry, 401(9), 2977-2985.

**[4]** Nelson, K. M., Dahlin, J. L., Bisson, J., Graham, J., Pauli, G. F., & Walters, M. A. (2017). The essential medicinal chemistry of curcumin: miniperspective. Journal of medicinal chemistry, 60(5), 1620-1637.

**[5]** Rouhani, S., Alizadeh, N., Salimi, S., & Haji-Ghasemi, T. (2009). Ultrasonic Assisted Extraction of Natural Pigments from Rhizomes of Curcuma Longa L. Progress in Color, Colorants and Coatings, 2(2), 103-113.

**[6]** Lestari, M. L., & Indrayanto, G. (2014). Curcumin. Profiles of drug substances, excipients, and related methodology, 39, 113-204.

**[7]** Kotra, V. S. R., Satyabanta, L., & Goswami, T. K. (2019). A critical review of analytical methods for determination of curcuminoids in turmeric. Journal of food science and technology, 56(12), 5153-5166.

**[8]** Chun, K.-S., Sohn, Y., Kim, H.-S., Kim, O. H., Park, K.-K., Lee, J.-M., Lee, J., Lee, J.-Y., Moon, A., Lee, S. S., & Surh, Y.-J. (1999). Anti-tumor promoting potential of naturally occurring diarylheptanoids structurally related to curcumin. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 428(1–2), 49–57.

**[9]** Tonnesen, H. H., & Karlsen, J. (1985). Studies on curcumin and curcuminoids. V. Alkaline degradation of curcumin. Zeitschrift für Lebensmittel-Untersuchung und-Forschung, 180(2), 132-134.

**[10]** Ravindran, P. N., Babu, K. N., & Sivaraman, K. (Eds.). (2007). Turmeric: the genus *Curcuma*. CRC press.

**[11]** Amalraj, A., Pius, A., Gopi, S., & Gopi, S. (2017). Biological activities of curcuminoids, other biomolecules from turmeric and their derivatives–A review. Journal of traditional and complementary medicine, 7(2), 205-233.

**[12]** Suhit et al., 2010 G. Suhit, K. Meghana, B. Ramesh, P. Anant Activity of water-soluble turmeric extract using hydrophilic excipients LWT – Food Science and Technology, 43 (2010), pp. 59-66

**[13]** Lukita-Atmadja, W., Ito, Y., Baker, G. L., & McCuskey, R. S. (2002). Effect of curcuminoids as anti-inflammatory agents on the hepatic microvascular response to endotoxin. Shock, 17(5), 399-403.

**[14]** Sharma, R. A., Gescher, A. J., & Steward, W. P. (2005). Curcumin: the story so far. European journal of cancer, 41(13), 1955-1968.

**[15]** Singh, S., & Khar, A. (2006). Biological effects of curcumin and its role in cancer chemoprevention and therapy. Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents), 6(3), 259-270.

**[16]** Bagheri, H., Ghasemi, F., Barreto, G. E., Rafiee, R., Sathyapalan, T., & Sahebkar, A. (2020). Effects of curcumin on mitochondria in neurodegenerative diseases. Biofactors, *46*(1), 5-20.

**[17]** Ramsewak, R. S., DeWitt, D. L., & Nair, M. G. (2000). Cytotoxicity, antioxidant, and anti-inflammatory activities of curcumins I–III from Curcuma longa. Phytomedicine, 7(4), 303-308.

**[18]** Song, E. K., Cho, H., Kim, J. S., Kim, N. Y., An, N. H., Kim, J. A., Lee, S.-H., & Kim, Y. C. (2001). Diarylheptanoids with free radical scavenging and hepatoprotective activity in vitro from *Curcuma longa*. Planta medica, 67(09), 876-877.

**[19]** Kalpravidh, R. W., Siritanaratkul, N., Insain, P., Charoensakdi, R., Panichkul, N., Hatairaktham, S., Srichairatanakool, S., Phisalaphong, C., Rachmilewitz, E., & Fucharoen, S. (2010). Improvement in oxidative stress and antioxidant parameters in β-thalassemia/Hb E patients treated with curcuminoids. Clinical Biochemistry, 43(4–5), 424–429.

**[20]** Mazumder, A., Raghavan, K., Weinstein, J., Kohn, K. W., & Pommier, Y. (1995). Inhibition of human immunodeficiency virus type-1 integrase by curcumin. Biochemical pharmacology, 49(8), 1165-1170.

**[21]** Anand, P., Thomas, S. G., Kunnumakkara, A. B., Sundaram, C., Harikumar, K. B., Sung, B., Tharakan, S. T., Misra, K., Priyadarsini, I. K., Rajasekharan, K. N., & Aggarwal, B. B. (2008). Biological activities of curcumin and its analogues (Congeners) made by man and Mother Nature. Biochemical Pharmacology, 76(11), 1590–1611.

**[22]** Nurfina, A. N., Reksohadiprodjo, M. S., Timmerman, H., Jenie, U. A., Sugiyanto, D., & Van der Goot, H. (1997). Synthesis of some symmetrical curcumin derivatives and their antiinflammatory activity. European journal of medicinal chemistry, 32(4), 321-328.

**[23]** Rasmussen, H. B., Christensen, S. B., Kvist, L. P., & Karazmi, A. (2000). A simple and efficient separation of the curcumins, the antiprotozoal constituents of *Curcuma longa*. Planta medica, 66(04), 396-398.

**[24]** Yodkeeree, S., Chaiwangyen, W., Garbisa, S., & Limtrakul, P. (2009). Curcumin, demethoxycurcumin and bisdemethoxycurcumin differentially inhibit cancer cell invasion through the downregulation of MMPs and uPA. The Journal of nutritional biochemistry, 20(2), 87-95.

**[25]** Nandakumar, D. N., Nagaraj, V. A., Vathsala, P. G., Rangarajan, P., & Padmanaban, G. (2006). Curcumin-artemisinin combination therapy for malaria. Antimicrobial agents and chemotherapy, 50(5), 1859-1860.

**[26]** Peram, M. R., Jalalpure, S. S., Joshi, S. A., Palkar, M. B., & Diwan, P. V. (2017). Single robust RP-HPLC analytical method for quantification of curcuminoids in commercial turmeric products, Ayurvedic medicines, and nanovesicular systems. Journal of Liquid Chromatography & Related Technologies, 40(10), 487–498.

**[27]** Stankovic, I. (2004). Curcumin: Chemical and Technical Assessment (CTA). JECFA*,* Rome, 8.

**[28]** Schieffer, G. W. (2002). Pressurized liquid extraction of curcuminoids and curcuminoid degradation products from turmeric (*Curcuma longa*) with subsequent HPLC assays. Journal of Liquid Chromatography & Related Technologies, 25(19), 3033-3044.

**[29]** Suresh, D., Manjunatha, H., & Srinivasan, K. (2007). Effect of heat processing of spices on the concentrations of their bioactive principles: Turmeric (*Curcuma longa*), red pepper (*Capsicum annuum*) and black pepper (*Piper nigrum).* Journal of Food Composition and Analysis, 20(3-4), 346-351.

**[30]** Kimbaris, A. C., Siatis, N. G., Daferera, D. J., Tarantilis, P. A., Pappas, C. S., & Polissiou, M. G. (2006). Comparison of distillation and ultrasound-assisted extraction methods for the isolation of sensitive aroma compounds from garlic (*Allium sativum*). Ultrasonics Sonochemistry, 13(1), 54-60.

**[31]** Vinatoru, M. (2001). An overview of the ultrasonically assisted extraction of bioactive principles from herbs. Ultrasonics Sonochemistry, 8(3), 303-313.

**[32]** Da Porto, C., & Decorti, D. (2009). Ultrasound-assisted extraction coupled with under vacuum distillation of flavour compounds from spearmint (carvone-rich) plants: comparison with conventional hydrodistillation. Ultrasonics Sonochemistry, 16(6), 795-799.

**[33]** Khan, M. K., Abert-Vian, M., Fabiano-Tixier, A. S., Dangles, O., & Chemat, F. (2010). Ultrasound-assisted extraction of polyphenols (flavanone glycosides) from orange (*Citrus sinensis* L.) peel. Food Chemistry, *119*(2), 851-858.

**[34]** Lianfu, Z., & Zelong, L. (2008). Optimization and comparison of ultrasound/microwave assisted extraction (UMAE) and ultrasonic assisted extraction (UAE) of lycopene from tomatoes. Ultrasonics Sonochemistry, *15*(5), 731-737.

**[35]** Adjé, F., Lozano, Y. F., Lozano, P., Adima, A., Chemat, F., & Gaydou, E. M. (2010). Optimization of anthocyanin, flavonol and phenolic acid extractions from Delonix regia tree flowers using ultrasound-assisted water extraction. Industrial Crops and Products, 32(3), 439-444.

**[36]** Shirsath, S. R., Sonawane, S. H., & Gogate, P. R. (2012). Intensification of extraction of natural products using ultrasonic irradiations—A review of current status. Chemical Engineering and Processing: Process Intensification, *53*, 10-23.

**[37]** Reverchon, E., & Osséo, L. S. (1994). Comparison of processes for the supercritical carbon dioxide extraction of oil from soybean seeds. Journal of the American Oil Chemists' Society, *71*(9), 1007-1012.

**[38]** Wang, L., & Weller, C. L. (2006). Recent advances in extraction of nutraceuticals from plants. Trends in Food Science & Technology, *17*(6), 300-312.

**[39]** Shen, Y., Han, C., Chen, X., Hou, X., & Long, Z. (2013). Simultaneous determination of three Curcuminoids in *Curcuma wenyujin YH chen et C. Ling*. by liquid chromatography–tandem mass spectrometry combined with pressurized

liquid extraction. Journal of Pharmaceutical And Biomedical Analysis, *81*, 146-150.

**[40]** Wakte, P. S., Sachin, B. S., Patil, A. A., Mohato, D. M., Band, T. H., & Shinde, D. B. (2011). Optimization of microwave, ultra-sonic and supercritical carbon dioxide assisted extraction techniques for curcumin from *Curcuma longa*. Separation and Purification Technology, 79(1), 50-55.

**[41]** Kwon, H. L., & Chung, M. S. (2015). Pilot-scale subcritical solvent extraction of curcuminoids from *Curcuma longa* L. Food chemistry, 185, 58-64.

**[42]** Kimthet, C., Wahyudiono, Kanda, H., & Goto, M. (2017, May). Extraction of curcumin from *Curcuma longa* L. using ultrasound assisted supercritical carbon dioxide. In AIP conference proceedings (Vol. 1840, No. 1, p. 100001). AIP Publishing LLC.

**[43]** Kiamahalleh, M. V., Najafpour-Darzi, G., Rahimnejad, M., Moghadamnia, A. A., & Kiamahalleh, M. V. (2016). High performance curcumin subcritical water extraction from turmeric (*Curcuma longa* L.). Journal of Chromatography B, 1022, 191-198.

**[44]** Kewen, T., Jianmin, Y., & Li, L. (2005). Microwave Assisted Extraction-Adsorption Separation of Curcumin from Turmeric. Chemical Industry and Engineering Progress, 24(6), 647.

**[45]** Bener, M., Özyürek, M., Güçlü, K., & Apak, R. (2016). Optimization of microwave-assisted extraction of curcumin from *Curcuma longa* L. (Turmeric) and evaluation of antioxidant activity in multi-test systems. Records of Natural Products, 10(5), 542.

**[46]** Rezaei, S., Najafpour, G. D., Mohammadi, M., Moghadamnia, A. A., Kazemi S. (2016). Formic acid and microwave assisted extraction of curcumin from turmeric (*Curcuma longa* L.). International Journal of Engineering, 29(2), 145-151.

**[47]** Sahne, F., Mohammadi, M., Najafpour, G. D., & Moghadamnia, A. A. (2016). Extraction of bioactive compound curcumin from turmeric (*Curcuma longa* L.) via different routes: A comparative study. Pak. Journal of Biotechnology, 13(3), 173-180.

**[48]** Sahne, F., Mohammadi, M., Najafpour, G. D., & Moghadamnia, A. A. (2017). Enzyme-assisted ionic liquid extraction of bioactive compound from

turmeric (*Curcuma longa* L.): Isolation, purification and analysis of curcumin. Industrial Crops and Products, 95, 686-694.

**[49]** Ou, Z. Q., Jia, L. Q., Jin, H. Y., Yediler, A., Sun, T. H., & Kettrup, A. (1997). Ultrasonic extraction and LC determination of linear alkylbenzene sulfonate in plant tissues. Chromatographia, *44*(7-8), 417-420.

**[50]** Shotipruk, A., Kaufman, P. B., & Wang, H. Y. (2001). Feasibility study of repeated harvesting of menthol from biologically viable menthaxpiperata using ultrasonic extraction. Biotechnology Progress, *17*(5), 924-928.

**[51]** Djilani, A., Legseir, B., Soulimani, R., Dicko, A., & Younos, C. (2006). New extraction technique for alkaloids. Journal of the Brazilian Chemical Society, 17(3), 518-520.

**[52]** Bong, P. H. (2000). Spectral and photophysical behaviors of curcumin and curcuminoids. Bulletin of the Korean Chemical Society, 21(1), 81-86.

**[53]** Santos, D. T., Veggi, P. C., & Meireles, M. A. A. (2012). Optimization and economic evaluation of pressurized liquid extraction of phenolic compounds from jabuticaba skins. Journal of Food Engineering, 108(3), 444-452.

**[54]** Mustafa, A., & Turner, C. (2011). Pressurized liquid extraction as a green approach in food and herbal plants extraction: A review. Analytica Chimica Acta, 703(1), 8-18.

**[55]** Zaibunnisa, A. H., Norashikin, S., Mamot, S., & Osman, H. (2009). An experimental design approach for the extraction of volatile compounds from turmeric leaves (*Curcuma domestica*) using pressurised liquid extraction (PLE). LWT-Food Science and Technology, 42(1), 233-238.

**[56]** Teo, C. C., Tan, S. N., Yong, J. W. H., Hew, C. S., & Ong, E. S. (2010). Pressurized hot water extraction (PHWE). Journal of Chromatography A, 1217(16), 2484-2494.

**[57]** Osorio-Tobón, J. F., & Meireles, M. A. A., (2013). Recent applications of pressurized fluid extraction: curcuminoids extraction with pressurized liquids. Food Public Health, *3*(6), 289-303.

**[58]** Mira, B., Blasco, M., Berna, A., & Subirats, S. (1999). Supercritical $CO_2$ extraction of essential oil from orange peel. Effect of operation conditions on the extract composition. The Journal of Supercritical Fluids, 14(2), 95-104.

**[59]** Zougagh, M., Valcárcel, M., & Rıos, A. (2004). Supercritical fluid extraction: a critical review of its analytical usefulness. TrAC Trends in Analytical Chemistry, 23(5), 399-405.

**[60]** Büyüktuncel, E. (2012). Gelişmiş ekstraksiyon teknikleri I. Hacettepe Üniversitesi Eczacılık Fakültesi Dergisi, (2), 209-242.

**[61]** Brunner, G. (2005). Supercritical fluids: technology and application to food processing. Journal of Food Engineering, 67(1-2), 21-33.

**[62]** Brunner, G. (1987). Stofftrennung mit ueberkritischen gasen (gasextraktion). Chemie Ingenieur Technik, 59(1), 12-22.

**[63]** Del Valle, J. M., & Aguilera, J. M. (1999). Revision: Extracción con CO2 a alta presión. Fundamentos y aplicaciones en la industria de alimentos/Review: High pressure $CO_2$ extraction. Fundamentals and applications in the food industry. Food Science and Technology International, 5(1), 1-24.

**[64]** Carabias-Martínez, R., Rodríguez-Gonzalo, E., Revilla-Ruiz, P., & Hernández-Méndez, J. (2005). Pressurized liquid extraction in the analysis of food and biological samples. Journal of Chromatography A, 1089(1-2), 1-17.

**[65]** Hawthorne, S. B., Yang, Y., & Miller, D. J. (1994). Extraction of organic pollutants from environmental solids with sub-and supercritical water. Analytical Chemistry, 66(18), 2912-2920.

**[66]** Liang, X., & Fan, Q. (2013). Application of sub-critical water extraction in pharmaceutical industry. Journal of Materials Science and Chemical Engineering, 1(05), 1.

**[67]** Ramos, L., Kristenson, E. M., & Brinkman, U. T. (2002). Current use of pressurised liquid extraction and subcritical water extraction in environmental analysis. Journal of Chromatography A, 975(1), 3-29.

**[68]** Smith, R. M. (2002). Extractions with superheated water. Journal of Chromatography A, 975(1), 31-46.

**[69]** Kubátová, A., Jansen, B., Vaudoisot, J. F., & Hawthorne, S. B. (2002). Thermodynamic and kinetic models for the extraction of essential oil from savory and polycyclic aromatic hydrocarbons from soil with hot (subcritical) water and supercritical $CO_2$. Journal of Chromatography A, 975(1), 175-188.

**[70]** Kaderides, K., Papaoikonomou, L., Serafim, M., & Goula, A. M. (2019). Microwave-assisted extraction of phenolics from pomegranate peels: Optimization, kinetics, and comparison with ultrasounds extraction. Chemical Engineering and Processing-Process Intensification, 137, 1-11.

**[71]** Zheng, X., Xu, X., Liu, C., Sun, Y., Lin, Z., & Liu, H. (2013). Extraction characteristics and optimal parameters of anthocyanin from blueberry powder under microwave-assisted extraction conditions. Separation and Purification Technology, 104, 17-25.

**[72]** Dandekar, D. V., & Gaikar, V. G. (2002). Microwave assisted extraction of curcuminoids from *Curcuma longa*. Separation Science and Technology, 37(11), 2669-2690.

**[73]** Destandau, E., Michel, T., & Elfakir, C. (2013). Microwave-assisted extraction. Natural Product Extraction: Principles and Applications, (21), 113.

**[74]** Kormin, F., Abdurahman, N. H., Yunus, R. M., & Rivai, M. (2013). Study the heating mechanisms of temperature-controlled microwave closed system (TCMCS). International Journal of Engineering Science and Innovative Technology (IJESIT), 2(5), 417-429.

**[75]** Li, M., Ngadi, M. O., & Ma, Y. (2014). Optimisation of pulsed ultrasonic and microwave-assisted extraction for curcuminoids by response surface methodology and kinetic study. Food chemistry, *165*, 29-34.

**[76]** Anonymous. 2020. Solvent Choice for Microwave Synthesis. CEM Corporation. Website: https://cem.com/en/microwave-chemistry/solvent-choice. Access Date: 10.02.2021.

**[77]** Priyadarsini, K. I. (2014). The chemistry of curcumin: from extraction to therapeutic agent. Molecules, 19(12), 20091-20112.

**[78]** Sevindik, O., & Selli, S. (2017). Üzüm Çekirdek Yağı Eldesinde Kullanilan Ekstraksiyon Yöntemleri. Gıda, 42(1), 95-103.

**[79]** Sowbhagya, H. B., & Chitra, V. N. (2010). Enzyme-assisted extraction of flavorings and colorants from plant materials. Critical Reviews In Food Science and Nutrition, 50(2), 146-161.

**[80]** Puri, M., Sharma, D., & Barrow, C. J. (2012). Enzyme-assisted extraction of bioactives from plants. Trends in Biotechnology, 30(1), 37-44.

**[81]** Zhang, G., Hu, M., He, L., Fu, P., Wang, L., & Zhou, J. (2013). Optimization of microwave-assisted enzymatic extraction of polyphenols from waste peanut shells and evaluation of its antioxidant and antibacterial activities in vitro. Food and Bioproducts Processing, 91(2), 158-168.

**[82]** Campbell, K. A., Vaca-Medina, G., Glatz, C. E., & Pontalier, P. Y. (2016). Parameters affecting enzyme-assisted aqueous extraction of extruded sunflower meal. Food Chemistry, 208, 245-251.

**[83]** Azmir, J., Zaidul, I. S. M., Rahman, M. M., Sharif, K. M., Mohamed, A., Sahena, F., Jahurul, M. H. A., Ghafoor, K., Norulaini, N. A. N., & Omar, A. K. M. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. Journal of Food Engineering, 117(4), 426–436.

**[84]** Kurmudle, N., Kagliwal, L. D., Bankar, S. B., & Singhal, R. S. (2013). Enzyme-assisted extraction for enhanced yields of turmeric oleoresin and its constituents. Food Bioscience, 3, 36-41.

# CHOCOLATE PRODUCTION, NUTRIENTS AND HEALTH BENEFITS*

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## ABSTRACT

Chocolate is defined as the final product formed by grinding the roasted cocoa beans with cocoa butter, and sugar and adding emulsifier and vanilla to this mixture. There are basically three types of chocolate: dark (bitter), milk and white. Dark chocolate consists of sugar, cocoa butter, and cocoa liquor. Milk chocolate consists of the same ingredients as dark chocolate but contains milk (milk powder). White chocolate, on the other hand, consists of sugar, cocoa butter and powdered milk, but no cocoa liquor. In this article dark chocolate production processes such as mixing and thinning, conching and tempering were investigated. Recent studies on nutrients and health benefits of chocolate were discussed as well.

***Key words**: Chocolate, Thinning, Conching, Tempering, Health benefits*

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## INTRODUCTION

Chocolate is the product formed by grinding the roasted cocoa beans and mixing them with cocoa butter and sugar, and adding emulsifier and vanilla to this mixture. Although the production of chocolate from cocoa beans is an industrial process, the stage from harvesting the cocoa seed to obtaining the bean takes place with a natural fermentation in the producing countries. Cocoa tree belongs to *Theobroma cacao*. Cocoa beans are in melon-like fruits, and during fermentation, this pectinaceous pulp surrounding beans is broken down and partially separated from the beans. One fruit contains about 20-22 cocoa beans **[1]**. Fermentation consists of a natural, seven-day microbial fermentation of the pulp at high temperatures of up to 50°C **[2]**. Some of the compounds that give flavor to cocoa are formed during this fermentation. Reduction of the amount of pectin by physical or mechanical means can also lead to an improved fermentation in reduced time **[2]**. The traditional fermentation is a result of a set of reactions catalyzed by a succession of microorganisms (yeasts, lactic acid bacteria, and acetic acid bacteria) that inoculate cocoa pulp spontaneously **[3]**. Finally, the fermentation end products formed, such as ethanol, lactic acid, and acetic acid kill the beans and cause production of flavor precursors **[2]**. Additionally, free amino acids, peptides and the inversion of sugars such as sucrose and the subsequent formation of reducing sugars occur **[3, 4]**. Cocoa is produced in different parts of the world, for example in Central and South America (Brazil, Ecuador, Peru, Papua New Guinea, Dominican Republic, Colombia, Venezuela, and Mexico), Africa (Ghana, Ivory Coast, Nigeria and Cameroon) and Asia (Indonesia, Malaysia, New Guinea). Cocoa beans contain a large amount of fat. It contains 50-54% fat, 10-15% protein, 4-5% moisture, 1% theobromine and 0.44% caffeine **[5]**. Cocoa butter consists of triglycerides formed by linoleic, oleic, palmitic and stearic acids with glycerol **[1]**. In this

article, the stages of the dark chocolate production process, the nutritional characteristics and health benefits of chocolate are examined in detail.

**Dark chocolate production**

According to the Turkish Food Codex, Cocoa and Chocolate Products Notification **[6],** dark chocolate is defined as a product consisting of cocoa products and sugar, containing at least 18% cocoa butter, 14% non-fat cocoa solid, and 35% total dry cocoa solids. Chocolate production flow chart is shown in Figure 1 **[7].** Dark chocolate production consists of five stages: mixing, refining, conching, tempering and crystallization (Figure 1). Each step in the production of chocolate has its own importance. In order to achieve the desired quality in chocolate, it is important to choose the right parameters in the production process as well as to use quality raw materials. In the refining process, it is aimed to reach the desired particle size with a three- or five-cylinder system and thus to obtain the desired smooth texture in the final product. This process also affects the rheological and sensory properties of the final product. Conching is accomplished by mixing the chocolate components at elevated temperatures on average >40°C. Conching time and temperature affect the texture, flavor and viscosity of chocolate **[8].** The crystalline network formed by lipids in the crystallization step affects some of the important physical and functional characteristics of chocolate (eg texture, crunching and shine). Tempering affects some other quality properties such as color, hardness and shelf life **[9].**

**Mixing and Refining**

The first step in chocolate production is to mix components such as sugar, cocoa liquor and cocoa butter into a paste. Mixer is shown in Figure 2. This mixture, which is prepared with an oil content of 8-24%, is refined to a particle size of less than 30 microns using two- and five-cylinder refiners. Particle size significantly affects the rheological and sensory properties of the final product **[7].** If the

particle size is larger than 30 microns, chocolate causes a rough texture in the mouth, and if it is smaller than 30 microns, it causes a paste-like texture [10].

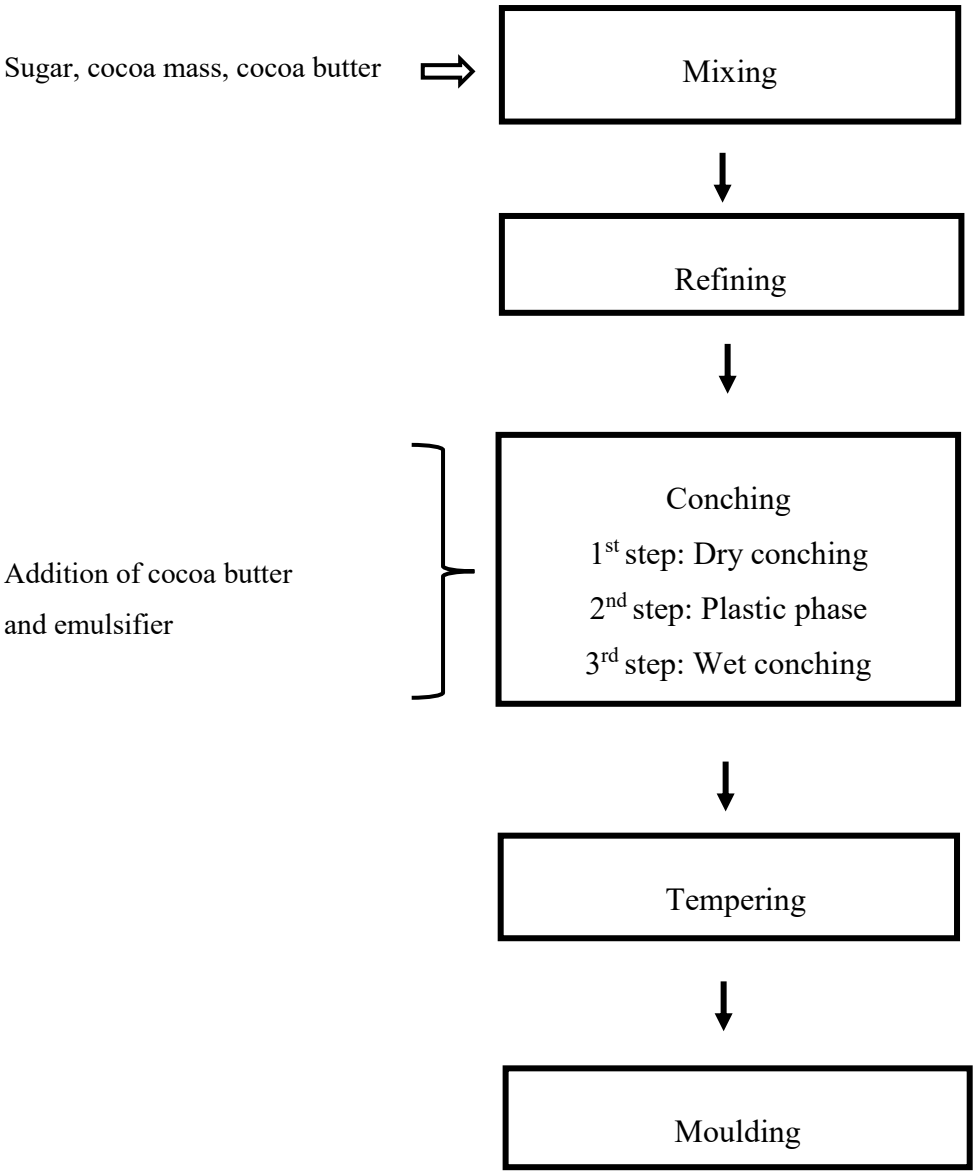

**Figure 1.** Chocolate production process **[7]**

**Figure 2**. Mixer

The five-cylinder (drum) refiner system consists of four vertically aligned hollow cylinders. Figure 3 shows the pilot scale refiner. The temperature control of these cylinders, which are held together by hydraulic pressure, is provided by the internal water flow. A thin layer of chocolate film is drawn into the accelerating drum and runs over the drum until it is separated by a scraper blade. The solid particles cut by the drum are coated with oil and form new surfaces and become active by absorbing volatile aroma compounds from cocoa components. Although the optimum particle size in dark chocolate varies according to the product and its composition, it should be as small as 35 microns. The refining process not only reduces particle size and prevents agglomeration, but also affects the dispersion of particles, each of which is coated with oil, throughout the continuous phase **[7].**

**Figure 3.** Pilot scale refiner

**Conching**

The refined mixture is subjected to conching, a process that contributes to the improvement of the taste, texture and viscosity of the final product **[7].** Conche is a scraper mixer that optimizes the flavor development of the final product and ensures that the chocolate mass becomes fluid. By mixing, the acidic flavors and moisture in the cocoa mass are reduced. Conching time is also important and plays an effective role on the texture, taste and quality characteristics of the final product **[10].** Conching time and temperature values vary depending on the type of chocolate. The average conching time in dark chocolate is 16-24 hours, and the temperature values can start from 70°C and increase up to 82°C. Cocoa butter and lecithin can be added towards the end of conching in order for the chocolate to reach the appropriate viscosity **[7].** In order to obtain a quality chocolate, it is required to go through three stages during conching: dry conching, plastic phase and wet conching. Figure 4 shows wet conching.

In the dry conching stage, the chocolate is in powder form and the moisture content is high. Too much moisture affects the fluidity of the product negatively. Since the surface of the chocolate powders is not completely covered with oil during the dry conching stage, this stage is very important to reduce the moisture

in the final product. At this stage, a chocolate with lower moisture content is produced by rapid heating and mixing of the powder. As the temperature rises,

**Figure 4.** Wet conching

the cocoa butter in the chocolate powder begins to melt and the particles stick together to form a paste-like structure. This stage constitutes the plastic phase of conching. Finally, oil and emulsifiers are added during the wet conching stage, ensuring that the chocolate has the right flow properties for the downstream processing steps **[11].**

**Tempering**

Cocoa butter may exhibit a series of crystallizations in polymorphic form as a function of triglyceride composition. The fatty acid composition affects the solidification of the oil and various physical crystallization conditions. Cocoa

butter has 6 polymorphic structures, they are expressed with Roman numerals from I to VI. The three basic polymorphic structures are α, β, β′. Form V (also called the β form) is the most desirable polymorphic form for a well-tempered chocolate, providing the desired brittleness and gloss in the product while also increasing its resistance to oil blooming **[12].** Tempering is very important for chocolate to be in a suitable polymorphic form and affects quality characteristics such as colour, hardness and shelf life. The tempermeter is shown in Figure 5. Tempering can take place in four basic steps. First, it is complete melting at 50°C, cooling to the crystallization point at 32°C, crystallization at 27°C, and transformation of all unstable crystals at 29–31°C **[12].**

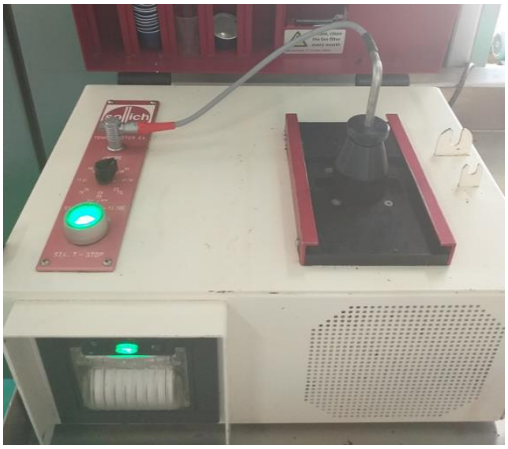

**Figure 5.** Tempermeter

Well-tempered chocolate should have good shape, colour, gloss properties and more stability, longer shelf life and harder properties. The reason why the tempering process differs in milk chocolate from dark chocolate is due to the different effects of milk fat in milk chocolate on crystal formation. In addition, the eutectic effect of milk fat has both an inhibiting effect on flowering and a softer texture and lower tempering temperature due to its lower melting point **[12].**

**Nutrients and Health Benefits of Chocolate**

The key ingredients in a chocolate formulation are; cocoa mass, cocoa butter, sugar and lecithin as emulsifier. However, the assortment in chocolate is the result of incorporating other ingredients such as nuts, dried fruits or cereals into formulations. The main categories of commercial chocolate are dark, milk and white chocolate, which differ in cocoa mass, milk fat and cocoa butter content **[13].** Typical nutritional values for different chocolate varieties are given in Table 1 **[11].** Chocolate and chocolate products, which have high energy and nutritional value, have an energy value of more than 3000 kcal/kg. Much of this high energy value comes from fat and carbohydrates. The most energy-rich component of chocolate is cocoa butter. Cocoa butter contains about 34% stearic acid, 34% oleic acid, 27% palmitic acid, all three of which are saturated fatty acids. Stearic acid has little effect on cholesterol levels. Oleic acid has a lowering or neutral effect on cholesterol levels. Palmitic acid, on the other hand, has moderate cholesterol-raising properties. Unsaturated fatty acids make up the rest of the cocoa butter, and cocoa butter contains almost no trans fatty acids **[11].** Chocolate also contains minerals, especially potassium, magnesium, copper and iron **[13].**

**Table 1.** Nutrition values for 100 g chocolate **[11].**

| | Dark | Milk | White |
|---|---|---|---|
| Energy (kcal) | 530 | 518 | 553 |
| Protein (gr) | 5 | 7 | 9 |
| Carbohydrate (gr) | 55 | 57 | 58 |
| Fat (gr) | 32 | 33 | 33 |
| Calcium (mg) | 32 | 224 | 272 |
| Magnesium (mg) | 90 | 59 | 27 |
| Iron (mg) | 3 | 2 | 0.2 |

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While most of the carbohydrates in chocolate come from sucrose, there are also small amounts of different carbohydrates in the dietary fibers found in cocoa. The type and amount of carbohydrates are important because of their effect on the glycemic index. The glycemic index is defined as the measure of the increase in blood glucose that occurs within two hours of consuming 50 grams of carbohydrates. Starchy products such as bread and potatoes are easily digested, causing a rapid rise in blood sugar. Conversely, since the amount of fat in foods slows the absorption of carbohydrates, the high fat content in chocolate causes blood sugar to rise more slowly, thus lowering the glycemic index **[11].**

The Aztecs and Mayans for many years enjoyed the use of cocoa beverages as beneficial for health. They believed cocoa products had therapeutic effects on diseases, as well. In recent years, scientific interest in the benefits of cocoa and chocolate products has increased again, especially in terms of reducing the risk of cardiovascular disease. Indeed, the positive effect of fat in the chocolate on cardiovascular diseases has been well documented **[14].** Recent research with a class of polyphenols and more specifically flavanols is relevant. Cocoa contains

epicatechin, catechin and procyanidin flavonols. It contains, polyphenols called tannins that contribute to chocolate's color and flavor. These tannins have been found to have anti-bacterial and anti-enzymatic activity. It reduces the formation of plaque on the teeth and prevents the formation of acid. Polyphenols are active compounds found found in a wide variety of foods, such as vegetables, fruits, tea, coffee, and red wine. Since dark chocolate contains more cocoa, the amount of polyphenols is higher than milky chocolate **[11].** Ten grams of dark chocolate contains approximately 120-150 mg of polyphenols **[12].**

It has been stated that cocoa flavonols can limit the progression of cardiovascular diseases by their anti-blood clotting, anti-inflammatory and antioxidant activities **[10, 11].** Flavanols can affect the reactivity of platelets and prevent blood clotting by reducing their tendency to agglomerate. In addition, flavonols have been observed to suppress some inflammatory reagents and increase anti-inflammatory nitric oxide. Studies have shown that cocoa and chocolate products cause an improvement in blood pressure and blood flow due to their effects on nitric oxide **[11].** In addition, regular consumption of dark chocolate has a protective effect against cardiovascular diseases in healthy individuals by reducing oxidative stress, decreasing arterial pressure and improving endothelial function **[11, 12].** There is the possibility that the inclusion of bioactive compounds found in chocolate in the diet may improve coronary circulation and ultimately reduce stroke and cardiovascular diseases. In a study by Fernández-Murga **[14]** and Fanton et al **[15],** the relationship between chocolate consumption and cardiovascular disease risk was investigated. It has been determined that chocolate consumption (<100 g/week) can reduce the risk of cardiovascular disease. However, it has been reported that excessive consumption of chocolate may cause adverse effects due to high sugar consumption, and approximately 45 g/week of chocolate is an appropriate dose **[10].**

Flavonols have an antioxidant effect against life-threatening diseases such as cancer, which helps prevent damage caused by free radicals in the body. It has been stated that the antioxidant activity produced by the consumption of dark chocolate, which is rich in flavonols, is higher than that of other antioxidant-rich foods and beverages such as green tea, red wine, blueberries and garlic **[10]**. Methylxanthines are a group of chemicals found in more than 60 plant varieties. Caffeine and theobromine compounds are found in significant amounts as methylxanthine in chocolate. It has been determined that caffeine stimulates the central nervous system. Cocoa also contains many other active compounds such as phenylethylamine, anandamide and tryptophan, all of which are thought to induce cravings for chocolate **[11].**

Chocolate consumption has positive physiological effects as well as psychological effects. Chocolate consumption can create an antidepressant effect by supporting the production of the neurotransmitter serotonin **[16].** In a study conducted with thirteen thousand six hundred and twenty-six (13.626) adults, the relationship between chocolate consumption and depression symptoms were evaluated and it was determined that dark chocolate consumption reduced the incidence of clinically depressive symptoms **[17]**. Also, another study showed that chocolate has a psychoactive property on improving stress and mood. In a study of three different types of chocolate (40g dark chocolate, 40g milk chocolate, 40g white chocolate) over two weeks, students who consumed dark or milk chocolate had reduced stress levels **[18]**. In a study conducted with 26 individuals who consumed 25 g of high polyphenol dark chocolate every day for 4 weeks, their mood and salivary cortisol levels were examined and a decrease in total daily cortisol, morning cortisol and cortisol/cortisone ratio was observed **[19].**

## REFERENCES

**[1]** Tokuşoğlu, Ö. (2015). Kakao Çikolata ve Çikolatalı Ürünler Bilimi ve Teknolojisi. Sidas yayınları, pp.232.

**[2]** Schwan, R. F. & Wheals, A. E. (2004). The microbiology of cocoa fermentation and its role in chocolate quality. Critical Reviews in Food Science and Nutrition, 44(4), 205-221.

**[3]** Munoz, M. S., Cortina, J. R., Vaillant, F. E., & Parra, S. E. (2020). Critical Reviews in Food Science and Nutrition, 60(10), 1593-1613.

**[4]** De Vuyst, L., and S. Weckx, S. (2016). The cocoa bean fermentation process: From ecosystem analysis to starter culture development. Journal of Applied Microbiology, 121(1), 5-17.

**[5]** Afoakwa, E. O., Quao, J., Takrama, J., Budu, A. S., & Saalia, F.K. (2013). Chemical composition and physical quality characteristics of Ghanaian cocoa beans as affected by pulp pre-conditioning and fermentation. Journal of Food Science and Technology, 50(6), 1097-1105.

**[6]** Türk Gıda Kodeksi Kakao ve Çikolata Ürünleri Tebliği, T.C. Resmî Gazete, 30229, Tebliğ No: 2017/293, Kasım 2017.

**[7]** Afoakwa, E. O., Paterson, A., & Fowler, M. (2007). Factors influencing rheological and textural qualities in chocolate – a review. Trends in Food Science and Technology, 18(6), 290-298.

**[8]** Konar, N., Özhan, B., Artık, N., Dalabasmaz, S., & Poyrazoglu, E. S. (2013). Rheological and physical properties of Inulin-containing milk chocolate prepared at different process conditions. CyTA- Journal of Food, 12(1), 55-64.

**[9]** Afoakwa, E. O., Paterson, A., Fowler, M., & Vieira, J. (2009). Influence of tempering and fat crystallization behaviours on microstructural and melting properties in dark chocolate systems. Food Research International, 42(1), 200-209.

**[10]** Rousseau, D. (2016). Microstructural Imaging of Chocolate Confectionery. In: Imaging Technologies and Data Processing for Food Engineers. (pp. 311-333). Springer, Cham.

**[11]** Beckett, S. T. (2008). The science of chocolate (2nd ed.). London, UK: Royal Society of Chemistry.

**[12]** Garti, N., & Aserin, A. (2012). Effect of Emulsifiers on Cocoa Butter and Chocolate Rheology, Polymorphism, and Bloom. In *Cocoa butter and related compounds* (pp. 275–305). AOCS Press.

**[13]** Torres-Moreno, M., Torrescasana, E., Salas-Salvadó, J., & Blanch, C. (2015). Nutritional composition and fatty acids profile in cocoa beans and chocolates with different geographical origin and processing conditions. Food Chemistry, 166, 125-132.

**[14]** Fernández-Murga, L., Tarín, J. J., García-Perez, M. A., & Cano, A. (2011). The impact of chocolate on cardiovascular health. Maturitas, 69(4), 312–321.

**[15]** Fanton, S., Cardozo, L. F. M. F., Combet, E., Shiels, P. G., Stenvinkel, P., Vieira, I. O., Narciso, H. R., Schmitz, J., & Mafra, D. (2021). The sweet side of dark chocolate for chronic kidney disease patients. Clinical Nutrition, 40, 15–26.

**[16]** Wollgast, J., & Anklam, E. (2000). Polyphenols in chocolate: is there a contribution to human health? Food Research International, 33(6), 449–459.

**[17]** Fanton, S., Cardozo, L. F. M. F., Combet, E., Shiels, P. G., Stenvinkel, P., Vieira, I. O., Narciso, H. R., Schmitz, J., & Mafra, D. (2021). The sweet side of dark chocolate for chronic kidney disease patients. Clinical Nutrition, 40, 15–26.

**[18]** Jackson, S. E., Smith, L., Firth, J., Grabovac, I., Soysal, P., Koyanagi, A., Hu, L., Stubbs, B., Demurtas, J., Veronese, N., Zhu, X., & Yang, L. (2019). Is there a relationship between chocolate consumption and symptoms of depression? A cross-sectional survey of 13,626 US adults. Depression and Anxiety, 36(10), 987–995.

**[19]** Al Sunni, A., & Latif, R. (2014). Effects of Chocolate Intake on Perceived Stress: A Controlled Clinical Study. International Journal of Health Sciences, 8(4), 397–406.

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