

1- INTRODUCTION

Essential oils have found wide fields of applications today in industry, they are used as ingredients in cosmetic, pharmaceutical, and food products due to their intense flavour (**Burt, 2004 and Bensmira *et al.*, 2007**).

Some of the essential oils available on the market are extremely expensive. The quality and the price of the essential oils depend on their qualitative and quantitative composition. As the demand of essential oils has increased in the last years, the supply has escalated as well. So, essential oils can be purchased nearly everywhere, for example, at pharmacies, drugstores or even local market places. With the increasing number of providers, the need for product control has also increased. Therefore, accurate and fast methods for the quality control of essential oils are necessary. Up to now, the standard method used for this purpose has been physicochemical and Gas Chromatography analysis combined with Mass spectrophotometry (GC/MS) (**Jirovetz *et al.*, 2003 and Asensio *et al.*, 2015**).

The determination of food authenticity and the detection of adulterated one had increasing importance to food industry especially in products of high commercial value such as essential oils. Partial substitution of such products by cheaper ingredients may lead to significant adulteration and may be of added economic benefits for adulterating persons.

The commercial designation of essential oils is not always an unequivocal indication of either source or quality. This is the case for different essential oils such as peppermint oils where there is not only a wide range of materials available but also a broad spectrum of application parameters. Due to the high price of peppermint oil, it is often adulterated with the cheaper mint oil, *M. arvensis* L. oil (**Spencer *et al.*, 1997**). However, in Egypt *M. arvensis* L. is not cultivated in commercial scale, whereas, the cultivated area with *M. spicata* (881 acre) is 5.6 folds higher than that cultivated with peppermint (156 acre) and the total production of the two herbs are 9736 and 3405 tons , respectively (**Ministry of Agriculture, 2006**). So, blending peppermint oil with spearmint oil will leads to an adulterated product but with low price. The quality and suitability for use of blended peppermint oil should be judged by odour sensory

analysis supported by the chromatographic analysis combined with mass spectroscopy (**Jirovetz *et al.*, 2002**).

Using GC-MS analysis for the qualitative and quantitative analysis of the volatile compounds is time – consuming, expensive and require highly skilled personnel. Potential alternative for quality assessment including vibrational spectroscopy which offers several advantages over conventional methods. It is fast, low-cost and requires minimum samples preparation (**Sandasi *et al.*, 2010**). Although vibrational spectroscopy has been embraced by many food industries (**Nurrulhidayah *et al.*, 2015**), the natural product industries are slowly adopting it in routine quality control procedures. Research on possible application in quality control of natural products, in particular essential oils, is making tremendous progress.

The use of essential oils to extend the shelf life of food products is becoming popular since consumers have become more conscious about potential health problems associated with synthetic preservatives (**Gómez-Estaca *et al.*, 2010** and **Holley and Patel 2005**). Previous studies with essential oils as food additives have revealed them to be advantageous, as observed by an increase in food shelf life (**Chouliara *et al.*, 2007** and **Jiang *et al.*, 2012**). The amount of essential oils, however, determines the acceptability of products since strong aromas might be imparted to the product. One alternative to the direct incorporation of essential oils in foodstuffs is active packaging where volatile compounds in the essential oil create a protective atmosphere, either antimicrobial or antioxidant (**Nerín *et al.*, 2006**).

The multivariate statistical analysis (MVA) including principal component analysis (PCA) for processing chromatographic data has been shown to be an efficient tool for classification, searching, similarities and detection of adulteration of essential oils with less expensive oil (**Mildner-Szkudlarz *et al.*, 2003**; **Biswas *et al.*, 2004**; **Capote *et al.*, 2007** and **Mildner-Szkudlarz and Jeleń 2008**) and it shows promise for routine quality control. However, as far as the authors are aware no study could be found concerning detection the adulteration of the essential oils by using MVA of the chromatographic data of the adulterated essential oils.

1.2- Aim of the work

The main objectives of the present study were:

- 1- To develop a technique based on volatile compound profiles comparison using MVA including PCA for detection of adulteration or assessment of the authenticity of essential oils as follows:
 - 1.1- Seven model samples containing peppermint oil mixed with different levels of spearmint oil were prepared and subjected to gas chromatography - mass spectrophotometry (GC-MS) analysis followed by PCA. A correlation between the odour quality of the adulterated model samples and the relative concentration of menthone and menthol, the potent odorants of peppermint oil was established.
 - 1.2- To evaluate authenticity of different ground cumin samples obtained from the local market at different location in comparison with authentic sample, by using PCA technique combined with chromatographic data.
- 2- To use FTIR spectroscopy based metabolite fingerprinting as a fast and direct determination of the adulteration of essential oils, alternative of the chromatographic analysis.
- 3- To evaluate the effect of baking and storage time on the stability of cinnamon bark oil used as exogenous flavour in biscuits. And to investigate its effect on the endogenous flavour generated in biscuits during baking and storage.

2- REVIEW OF LITERATURE

2.1- Essential oils

Essential oils are aromatic and volatile liquids extracted from plant material, such as flowers, roots, bark, leaves, seeds, peel, fruits and wood (**Sanchez *et al.*, 2010**). The worldwide market for essential oils has been estimated at 2.6 billion \$ US with an annual growth rate of 7.5%. Essential oils have been used for centuries in medicine, perfumery and cosmetics, and have been added to foods as part of spices or herbs. Their initial application was in medicine, but in the nineteenth century, their use as aroma and flavour ingredients increased and became their major use. Almost 3000 different essential oils are known and 300 are used commercially in the flavour and fragrances market (**Burt, 2004**). Essential oils are considered to be secondary metabolites and important to plant defense as they often possess antimicrobial properties (**Tajkarimi *et al.*, 2010**).

Usually the fragrance and flavour substances are isolated by hydro-distillation from the dried or fresh plant material (e.g. leaves, seeds, fruits, stems, bark or wood). Most of the oils are used in perfume compositions as well as for flavouring of food-stuffs or mouth care products. Some essential oils containing high phenol content such as thyme and oregano oil are also used in phytopharmaceutical products or as additives for pet food relating to their antibiotic properties.

Several volatile plant components are thermolabile, sensitive to acids or easily hydrolyzed, whereas the composition and quality of the obtained essential oils depends not only on the individual raw material but also on the distillation procedure applied. The constituents of the essential oils are mainly monoterpenes and sesquiterpenes, which are hydrocarbons with the general formula $(C_5H_8)_n$. Oxygenated compounds derived from these hydrocarbons, include alcohols, aldehydes, esters, ethers, ketones, phenols and oxides.

In order to ensure the quality of essential oils used in fragrances, cosmetic materials and aroma preparations, usually gas chromatography combined with flame ionization or mass spectrometry detection is applied. Today, a large number of commercial essential oils have been described by the

International Organization for Specification (ISO), the Food Chemical Codex and the European Pharmacopoeia (**European Pharmacopoeia, 1983 and FCC, 1996**). During the last decades, the economic importance of essential oils has led to systematic breeding activities; in this context numerous new cultivars were obtained by alternation of generations or by vegetative routes. Higher yields of essential oil and particularly changed profiles of the oil are primary targets, leading to deviations from the standardized product quality.

In Egypt *Mentha* spices, cumin seeds and cinnamon bark are among the most commercially important aromatic plants. Their quality mainly attributed to the essential oil.

2.1.1- *Mentha* spices

Mentha, commonly known as mint, is one of the most common herb which has been known for its medicinal and aroma-therapeutic properties since ancient times. The ancient Egyptians, Greeks and Romans used mint as flavouring agents for food and as medicine (**Kumar et al., 2011**). The essential oils of mint have been used as perfumes, food flavours, deodorants, pharmaceuticals and insecticides (**Kumar et al., 2011**), antibiotic (**Emami et al., 2012**), antimicrobial (**Yadegarinia et al., 2006**) and antioxidant (**Kizil et al., 2010**). *Mentha* is the most important genus in *Lamiaceae* family because it contains a number of taxa, the essential oils of which have a high economic value. The amount of the oils produced annually is over 23,000 metric tons with a value exceeding 400 million \$ (**Lawrence, 2006**). This makes them the most economically important essential oils produced.

The genus *Mentha* includes five sections and 25- 30 species (**Harly and Brighton, 1977**). However, in Egypt *Mentha piperita* L. (*M. piperita*) and *Mentha spicata* L. (*M. spicata*) are the most widely cultivated for the commercial oil production (**Kumar et al., 2011**).

Peppermint (*M. piperita*) oil is one of the most popular and widely used essential oils, mostly because of its main components menthol and menthone (**Gul, 1994 and Díaz-Maroto et al., 2008**). In peppermint leaves, *trans*- p-menthone and l-(-)-menthol were the major components, followed by *trans*-methyl acetate, 1, 8-cineole and limonene, *trans*-sabinene hydrate and *cis*-p-menthone (**Díaz-Maroto et al., 2008**). Menthol is a waxy, crystalline

substance, has a pleasant taste. It is used for various medical purposes while menthone is used in perfumery and as a flavour agent. Normally, essential oil of *M. piperita* has 30 - 55% of menthol while menthone is between 14 and 32% (**ESCOP, 1997**). International demand for peppermint oil has increased in the past few years.

Among the peppermint of different origins studied, peppermint of USA and Egypt origin contains the highest menthol and gives optimum oil yield (**Kumar et al., 2011**). **Fig. (1_a)** shows the formula structures of the main volatile compounds in *M. piperita* oil.

Spearmint (*M. spicata*) is intensively cultivated for its essential oil. The leaves, herbs and essential oil of *M. spicata* were used much earlier than those of peppermint (**Hornok, 1992**). The ground fresh biomass and dried leaves of the plant are used as spice and herbal tea, and cultivated commercially in the entire world. Spearmint oil has also economic importance and is used in perfumery, confectionary and pharmaceutical preparations. Carvone is the main component of the essential oil of *M. spicata*, for which it is widely used as spices. It constitutes 50 - 65% of its total monoterpenes composition (**Kokkini et al., 1995**). Other major components of *M. spicata* oil (**Fig. 1_b**) are limonene, and 1, 8- cineole (**Kokkini et al., 1995 and Telci et al., 2010**). The composition of essential oil of *M. spicata* has been extensively investigated. For example, carvone and menthone – rich oils, carvone and neodihydrocarveol – rich oils (**Nagasawa et al., 1976a,b**), dihydrocarveol and carvone – rich oils (**Sivropoulou et al., 1995**), carvone and linalool – rich oils (**Hadjiakhoondi et al., 2000**), high carvone – rich oils and lower limonene – rich oils (**Edris et al., 2003 and XiaoHua et al., 2012**).

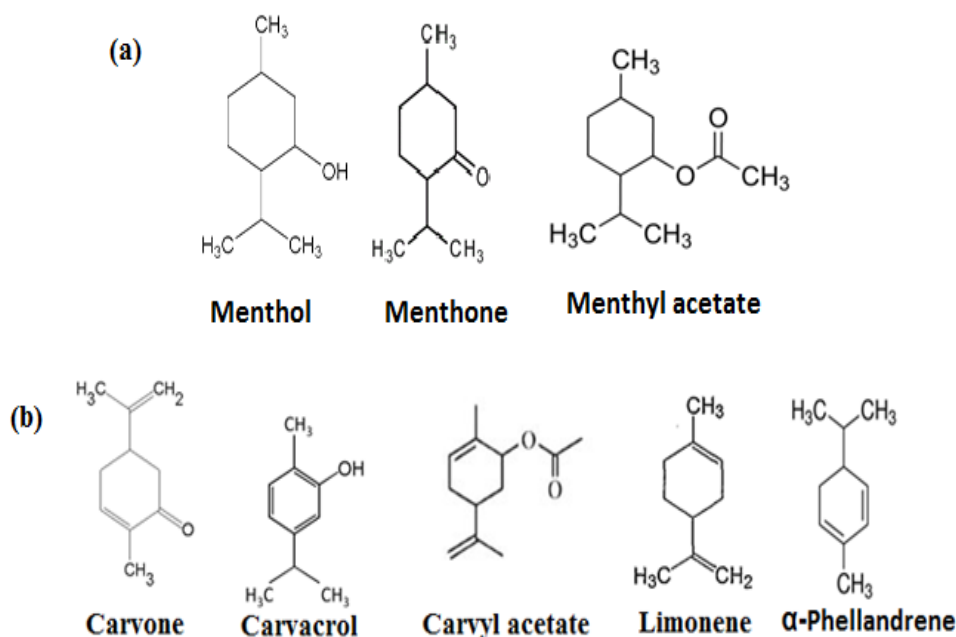


Fig. (1): Formula structures of the main volatile compounds in two *Mentha* spices *M. piperita* oil (a) and *M. spicata* oil (b).

2.1.2- Cumin seed

Cumin (*Cuminum cyminum* Linn.), an important commercial seed spice belonging to the *Apiaceae* family, is valued for its aroma, medicinal and therapeutic properties (Sowbhagya *et al.*, 2008).

Cumin is a small annual and herbaceous plant belonging to the *Apiaceae* family. It is one of the popular spices regularly used as a flavouring agent. It is cultivated in Arabia, India, China and in the countries bordering the Mediterranean Sea (Thippeswamy and Naidu 2005). Today, cumin is the second most popular spice in the world after black pepper. Cultivation of cumin requires a long, hot summer of 3–4 months, with daytime temperatures around 30°C; it is drought tolerant, and is mostly grown in Mediterranean climates. It is grown from seed, sown in spring, and needs fertile, well-drained soil (Hajlaoui *et al.*, 2010). Cumin seeds are used as a spice for their distinctive aroma, popular in Indian, Pakistan, North Africa, Middle East, Sri Lankan, Cuban, Northern Mexican cuisines and the Western

Chinese cuisines of Sichuan and Xinjiang (**Daniel and Maria 2000**). *C. cyminum* seeds have been used for treatment of toothache, dyspepsia, diarrhoea, epilepsy and jaundice (**Nostro et al., 2005**). The proximate composition of the seeds indicates that they contain fixed oil (approximately 10%), protein, cellulose, sugar, mineral elements and volatile oil (**Li and Jiang 2004**). Cumin seeds contain volatile oil (1–5%) that imparts the characteristic aroma to the seeds. The composition of *C. cyminum* essential oil has been the subject of previous studies (**Li and Jiang 2004; Oroojalian et al., 2010; Hajlaoui et al., 2010 and Rebey et al., 2012**).

Cumin seed essential oils are pale to colorless depending on age and regional variations. The ripe seeds of cumin are used for essential oil production, both as whole seeds or coarsely ground seeds. If freely alcohol-soluble oil is required, the whole seed must be used. Hydro-distillation is used for essential oil extraction, producing a colorless or paleyellow oily liquid with a strong odour (**Rebey et al., 2012**).

Sowbhagya et al. (2008) evaluated the effect of size reduction and expansion on the yield and quality of cumin seed oil. For small batch size operations (200g), oil yield was found to be the same (3.4%) for both ground and flaked samples. However, in the operations of larger batch, flakes resulted in significantly higher (3.3%) oil yield as compared to ground samples (2.8%) indicating the advantage of flaking over grinding. The aqueous portion of the distillate in both cases had an equal proportion of volatile oil (0.2%). Flavour profiles of the volatile oils revealed that retention of lower boiling terpene compounds and character impact compound, cuminaldehyde were higher in oil obtained from flakes as compared to powder.

The essential oil is responsible for the characteristic cumin odour (**Peter, 2001**). The odour and flavour is due principally to the aldehydes present. Studies of the chemical composition of cumin oil from different countries showed the presence of the following components: α -pinene (0.5%), myrcene (0.3%), limonene (0.5%), 1-8-cineole (0.2%), p-menth-3-en-7-ol (0.7%), p-mentha- 1, 3-dien-7-ol (5.6%), caryophyllene (0.8%), β -bisabolene (0.9%), β -pinene (13.0%), P-cymene (8.5%), β -phellandrene (0.3%), γ -terpinene (29.5%), cuminic aldehyde (32.4%), cuminyl alcohol

(2.8%), β -farnesene (1.1%) together with far smaller quantities of α -phellandrene, α -terpinene, cis and trans sabinene, myrtenol, α -terpineol and phellandral. Other studies show that cumin essential oil mainly contains monoterpene aldehydes. The major compounds include cumin aldehyde (p-iso propyl benzaldehyde, 25 to 35%), terpinene (29.5%), α - and β - pinene (21%), p-cymene (8.5%), p-mentha-1,3-dien-7-al (5.6%), cuminy alcohol (2.8%) and β -farnesene (1.1%). **Fig. (2)** shows the formula structure of the main volatile compounds in cumin seed oil.

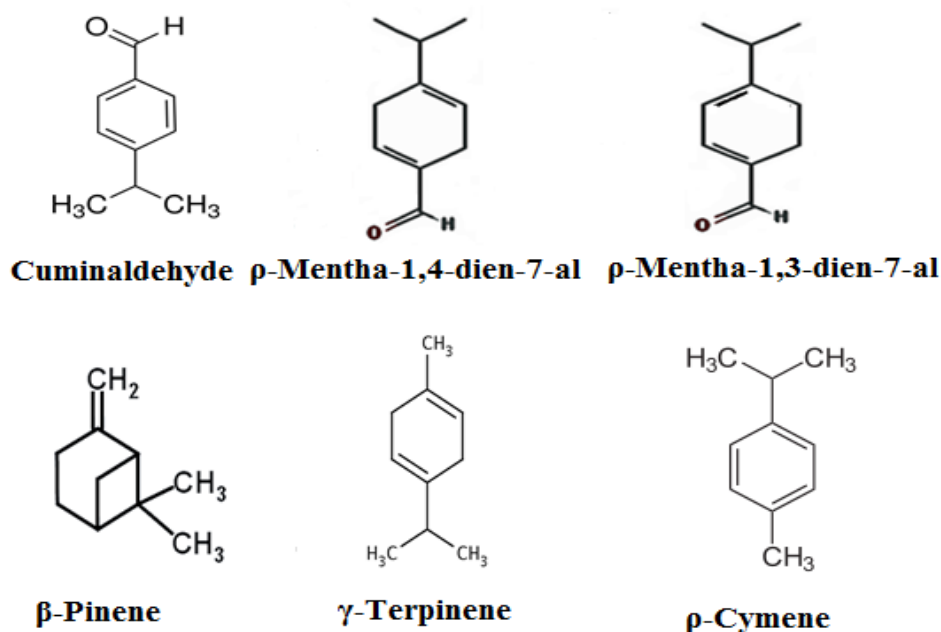


Fig. (2): Formula structures of the main volatile compounds in cumin seed oil.

2.1.3- Cinnamon bark

Cinnamon (*Cinnamon cassia*) has been used as a spice for thousands of years; several references to it are found in the Bible. In Egypt, cinnamon was a spice used in embalming fluid. A traditional use of cinnamon as herb includes application as an astringent, germicide and antispasmodic. Cinnamon was one of the early treatments for chronic bronchitis (**Ziment, 1991**). Other traditional uses include the treatment of impotence, frigidity,

dyspnea, inflammation of the eye, leucorrhea, vaginitis, rheumatism and neuralgia, as well as wounds and toothaches (**WHO, 1999**).

The flavouring agent cinnamaldehyde in cinnamon oil is added to toothpaste to mask the taste of pyrophosphate, which is an unpleasant tasting compound that inhibits plaque calcification by interrupting the conversion of amorphous calcium phosphate to hydroxyapatite. The skin sensitizing properties of cinnamon bark oil limits use of this essential oil in cosmetic and other topical products (**Hartmann and Hunzelmann 2004**).

Essential oils are important components of cinnamon bark, and these oils contain large quantities of terpenes and aromatic compounds. Specifically, cinnamaldehyde is the primary component of cinnamon oils (**China Pharmacopeia Commission, 2010**). Cinnamon has been identified to possess excellent anti-inflammatory activity (**Chao *et al.*, 2005**), antioxidant (**Mancini-Filho *et al.*, 1998** and **Murcia *et al.*, 2004**), anticancer (**Shan *et al.*, 1999** and **Schoene *et al.*, 2005**) and antibacterial properties (**Chang *et al.*, 2001**). Currently, cinnamon is widely used in pharmaceutical preparations, seasonings, cosmetics, foods, drinks, commodity essences and chemical industries, and it has become one of the most important natural plant resources and has a significant economic value.

Cinnamon essential oil is well known for its antimicrobial activity (**López *et al.*, 2005**; **Rodríguez *et al.*, 2008** and **Kim *et al.*, 2015**). It has also been reported to have antioxidant activity, derived mostly from its phenolic components (**El-Baroty *et al.*, 2010**). As for many other free radicals, OH can be neutralized if a hydrogen atom is provided. The scavenging activities of phenolic substances may be due to hydrogen donation during hydroxyl substitutions (**Mathew *et al.*, 2006**). For this reason, antioxidant behaviour is associated with phenolic compounds. Cinnamaldehyde (**Fig. 3**) is the major volatile components in cinnamon bark essential oil followed by eugenol and β -caryophyllene (**Tomaino *et al.*, 2005**).

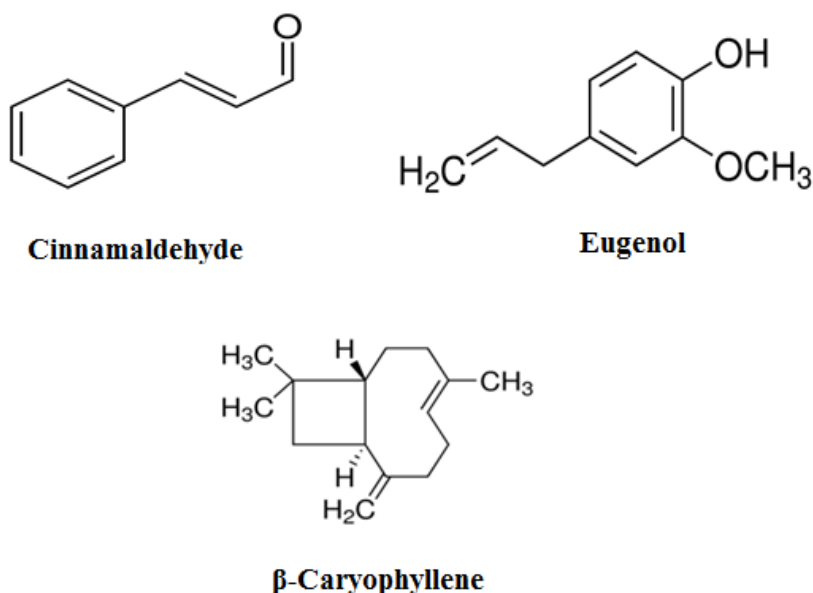


Fig. (3): Formula structures of the main volatile compounds in cinnamon bark oil.

2.2- Effect of baking and storage on endogenous and exogenous flavour in biscuits

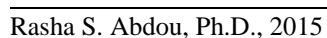
Food flavouring is widely used in baked products to reinforce or to generate a desired aromatic note. Nevertheless in the initial elaboration steps (incorporation of the aroma in the dough, kneading, etc.) and mainly during baking significant losses of the added aroma compounds will occur (Reineccius and Whorton 1990; Brauss *et al.*, 1999; Heiderich and Reineccius, 2001 and De Roos and Mansencal, 2003). Flavour loss during baking can be mainly explained by the volatility of the flavour compounds in the matrix and the amount of water loss during the heating process (Reineccius and Whorton 1990 and De Roos and Graf 1995). As most of the flavour compounds are hydrophobic, fat content highly influences flavour loss (Reineccius and Whorton 1990; Clawson *et al.*, 1996; Brauss *et al.*, 1999; De Roos, 2003 and De Roos and Mansencal 2003). In order to quantify the aroma compounds added to the baked cereal matrices or the compounds generated during baking, many different techniques for the

isolation of aroma compounds have been proposed, but the most widely used are direct extraction with organic solvents (**Brauss *et al.*, 1999; De Roos and Graf 1995 and De Roos and Mansencal, 2003**) and simultaneous steam distillation extraction (SDE) (**Whorton and Reineccius 1989; Zehentbauer and Grosch, 1998 and Kirchhoff and Schieberle 2002**).

During baking, there are a large number of complex interacting chemical reactions and physical processes that lead to the generation of the final biscuit flavour. Chemical processes include Maillard chemistry, caramelisation and lipid oxidation (**Ait Ameer *et al.*, 2008**). Physical processes may include volatilisation, physical binding, physical entrapment and the localisation and redistribution of reactants and aroma compounds across the matrix (**De Roos, 2006**). The broad range of these reactions makes the prediction of the final chemical composition of baked products complex, although through retrospective analysis, key physical and chemical traits can be understood and managed.

The reaction of amino acids and carbohydrates at elevated temperatures, compounds with antioxidant effect are generated as a result of non-enzymatic browning process (Maillard-reaction). Mechanisms of this reaction are well known because of their importance to the colour and flavour in cooked and processed food products (**Davies and Labuza 1997**). The first step involves the addition of the carbonyl group of the open-chain form of a reducing sugar to the primary amino group of an amino acid, peptide or other compound with a free amino group (**Fig. 4**). Sugar sources can include dextrose, fructose, and high fructose corn syrup, among others (**Davies and Labuza 1997**). The elimination of water yields a Schiff base that cyclizes to give the corresponding N – substituted aldosylamine, which is converted to the 1-amino-1-deoxy-2-ketose (Amadori product). When a ketose is involved instead of an aldose sugar, then a ketosylamine is formed that undergoes a Heyns rearrangement to form a 2-amino-2-deoxyaldose (Heyns product). These compounds do not contribute to flavour directly but they are important precursors of flavor compounds (**Whitfield, 1992**). The Amadori products are transformed by different pathways depending on the conditions. Dehydration produces furfural (from a pentose) or 5- hydroxyl methyl furfural or 5-methylfurfural from a hexose or reductones and dihydroreductones that give different furan derivatives. Dihydroreductones can also give, by Strecker reaction (oxidative deamination and

Fig. (4): Principal ways for the formation of flavour compounds during the Maillard reaction.



The interactions between the products of the Maillard reaction and those of the Strecker degradation lead to the formation of many important classes of flavor compounds; these include heterocyclic compounds such as pyrazines, oxazoles, thiophenes, and heterocyclic compounds with more than one sulfur atom. The Maillard reactions occurring during the baking process are the main chemical events responsible for the generation of compounds with low odor thresholds associated with the roasty pleasant aromatic notes in bakery products, and leads to the formation of coloured compounds as well as a large number of volatile aroma compounds like pyrazines which are responsible for typical nutty and crust odours (**Maarse, 1991**). These notes are highly desirable and intimately associated in consumer's minds with a delicious, high-grade product (**Schieberle and Grosch 1985; Rychlik and Grosch 1996 and Kirchhoff and Schieberle 2002**).

Aroma compounds present in solid foods are, by definition, volatile and therefore liable to be lost through volatilisation. In addition, aroma compounds may react with themselves, interact with structures within a product (**Fernández-Vázquez *et al.*, 2013**) or other ingredients (**Kant *et al.*, 2004; Tietz *et al.*, 2008; Fisk *et al.*, 2011 and Fisk *et al.*, 2012**), oxidative degradation may occur, as initiated by free radicals, metal ions or oxygenated species (**Frankel, 1985 and Chapman *et al.*, 1998;**), and migration between phases of the food matrices (**Given, 2009**) and the product headspace may occur to varying degrees (**Fisk *et al.*, 2012**) across different time points (**Yu *et al.*, 2012**).

2.2.1- Flavouring of baked products with aromatic plants

Natural aromatic plants and spices have been widely used in many food products such as meat and meat products, dairy and bakery products (**Pokorny, 1991**). The keeping quality of baked foods such as crackers, cookies and biscuits is of great economic importance since these products are widely used and are often stored for extended periods before consumption. The spices most commonly used in bakery products are cinnamon, mint, nutmeg, mace cloves, poppy and sesame seeds (**Bassiouny *et al.*, 1990**).

2.2.2- Effect of storage

During storage, the more mobile components of a food may interact with the packaging, which may lead to changes in the concentration of flavour precursors, desirable food flavours and off-notes. During product ageing, the process of lipid oxidation will lead to the generation of a wide range of

hydroperoxides which can then degrade to form secondary oxidation products. In the example of linolenic acid, volatile secondary products such as 2,4-decadienal (**Ullrich and Grosch 1987**) and 2,4-heptadienal (**Dixon and Hammond 1984**) may be formed and can therefore be used as marker compounds for the progression of lipid oxidation. These compounds were found in foods with oxidized and stale flavours (**Saison *et al.*, 2009**), and can contribute an oily off-note (**Josephson and Lindsay 1987**).

The effect of time and temperature on the quality of Lemon balm (*Melissa officinalis* L.) essential oil was investigated. The essential oil of air dried samples was obtained by hydro-distillation and was analysis by GC and GC-MS (**Najafian, 2014**). Changes in essential oil compositions were detected during storage for four months in refrigerator (4°C), freezer (-20°C), and at room temperature. The results indicated that, at room temperature the proportion of compounds with lower boiling temperatures such as citronellal (25.8 – 12.6 %), neral (18.9- 4.0%) and geranial (27.0- 4.6%) decreased. Furthermore, the oil compositions showed the least alterations and *M. officinalis* kept its primary quality when stored at low temperatures, particularly at -20°C.

Yang *et al.* (2013) examined the influence of flavour solvent (PG or TA), used as solvent delivery of vanillin flavour, on the chemical and physical stability and key sensory attributes of shortcake biscuits. The specific objective of this study was to explain the interaction of flavour solvent and time (ageing) on the stability of added vanillin. The author concluded that choice of flavour delivery solvent in shortcake biscuits can have a significant impact on measured fracturability, the loss of aroma compounds, concentration of HMF and the progression of lipid oxidation in fresh products and during ageing.

2.2.2- Encapsulation of exogenous flavour

Loss of added aroma compounds will produce a global decrease in the aromatic intensity of the product and a distortion of its final aromatic note (**Varadachari, 2002**), as well as economic loss for the food industry. It is, therefore, important to favour retention of the aroma in the baked cereal matrices in order to preserve the initial added aroma or to facilitate the retention of the molecules generated during baking. Encapsulation