CZECH UNIVERSITY OF LIFE SCIENCE PRAGUE

Faculty of Tropical AgriSciences

Analysis of Influence of Essential Oil Blanching on Drying Kinetics of Dehydrated Meat

Master Thesis

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Declaration

I, Diego Antonio Claramount Ruiz, declare that this thesis, submitted in fulfillment of requirements for the MSc. degree, at Faculty of Tropical Agriculture of the Czech University of Life Sciences Prague, is wholly my own work unless otherwise referenced or acknowledged.

In Prague 21.04. 2016

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Abstract

Meat is one of the most valuable livestock products, for many people serves as their first source of animal protein. Rising demand for meat in developing countries combined with poor access to synthetic antioxidants necessary for proper food preservation along with their potential side-effects highlights the importance of feasible alternatives for rural populations.

Among the most critical components that cause quality deterioration are the lipids and proteins oxidation, in time if not treated properly this will cause off-odor and off-flavor production in the meat, for that reason this dissertation focus on the application of Essential Oil (EO) as alternative to those more commonly used synthetic antioxidants like butyl hydroxyanisole (BHA) and butylhydroxytoluene (BHT) as pre-treatment of dehydrated meat, and its further effects if any during the drying process.

Beef meat samples were prepared, and tree different types of pretreatment were studied along with a combination of three possibilities as concentration of Essential Oil, the three types of pre-treatment used were; Steam Blanching (SB), Hot Air Blanching (HAB) and Oil Treatment (OT), this were combined with different concentrations of Oregano EO, those were 1.5ml, 3ml, and 6ml. The approach was to find if there was any correlation between the concentration of Oregano EO used versus the speed of drying and if there was any correlation between the different types of pretreatment applied.

It was found depending on the pre-treatment applied the concentration of Oregano EO might influence the drying process in a way in which it will make it faster or totally the opposite, as it was found with the Hot Air Blanching (HAB) and Oil Treatment (OT) pre-processes respectively, on the other hand it was found that no pattern is followed on the Steam Blanching process meaning that the different concentrations of Oregano EO used makes no difference ($p<0.05$) during the drying process. In conclusion, this study found that there was difference in the speed of drying, but this is not always due to the usage of Oregano EO, and also that depending on the pre-treatment used the usage of Oregano EO might do the drying go faster or sometimes also slower it down.

Key Words: Oregano, Essential Oil, Antioxidants, Drying Process, Pre-Treatment.

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1. Preface

Meat is one of the most valuable products around the world; the meat consumption is increasing in the developing countries most of it thanks to a positive economic growth, encouraging those new members of the middle class to change their consumption habits towards more consumption of meat for instance.

Consumption of meat per capita increase from 10 kg in the 1960's to 26 kg in 2000 and is forecast to reach 37 kg by 2030 (Heinz and Hautzinger, 2007), meaning that it will increase by more than 50%, thus adequate and feasible alternatives for proper storage and conservation is required to assure food supply and reducing its loss due to incorrect storage procedures.

The lack of studies on the field in regards to the usage of Essential Oil in dehydrated meat, added to the importance to assure proper meat supply for the coming years are the main drivers of this thesis.

One of the most widely used techniques to preserve food is drying processes, this basically means preservation of food for indefinite periods by extracting the moisture, thereby inhibiting the growth of microorganism, additional pre-treatments are given to the drying procedure to enhance drying and speed and to avoid growing of microbiological organisms by evaporating water from the product. All this is to assure a proper and healthy storage of food.

2. Literature Review

2.1 Drying of Meat

Even on developing countries the consumption of meat is increasing, and despite the fact that there are still differences among them when it comes to consumption, it could be easily seen that its increasing step by step (see Fig.1), for that reason we have to take on to consideration that, meat is a highly perishable product and soon becomes unfit to eat and possibly dangerous to health through microbial growth, chemical change and breakdown by endogenous enzymes (Bender, 1992)

One of the most widely used techniques to preserve food is drying processes, this basically means preservation of food for indefinite periods by extracting the moisture, thereby inhibiting the growth of microorganism, additional pre-treatments are given to the drying procedure to enhance drying and speed and to avoid growing of microbiological organisms by evaporating water from the product. All this is to assure a proper and healthy storage of food.

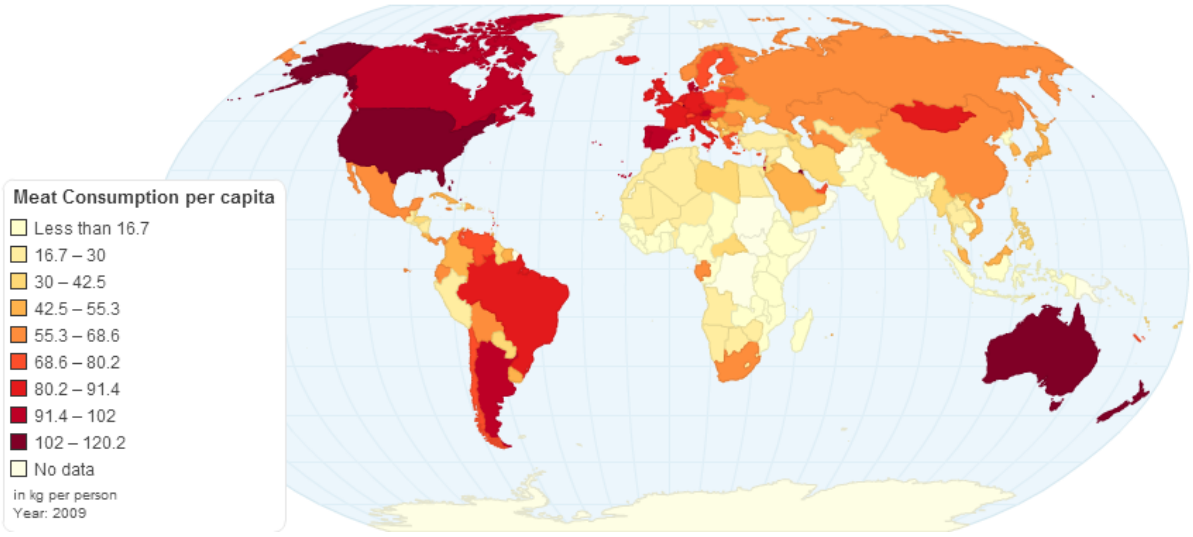

Fig.1 Current Worldwide Annual Meat Consumption per capita (FAO, 2013)

The water can be found in three different states, solid, liquid and gas. The phase in which it will be found it will depend on the temperature and pressure conditions and this can be seen in the phase diagram presented bellow (see Fig. 2)

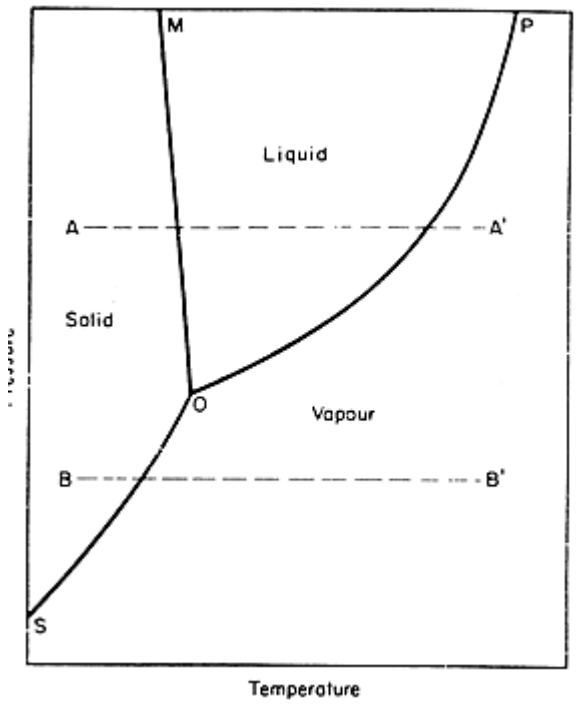

Fig.2 Water Phase Diagram (Mogk, 2013)

If heat is applied to water in any state at constant pressure, the temperature rises and the condition moves horizontally across the diagram, and as it crosses the boundaries, a change of state will occur. For example, starting from condition A on the chart adding heat warms the ice, then melts it, then warms the water and finally evaporates the water to condition A'. Starting from condition B, situated below the triple point, when heat is added, the ice warms and then sublimes without passing through any liquid state. (Earle, 1983)

Drying processes fall into three categories:

- Air and contact are drying under atmospheric pressure. On this case, the heat is transferred through the foodstuff either from the heated air or from heated surfaces. The vapor is removed by air.

- Vacuum drying. In vacuum drying, taking advantage of the fact that evaporation of water occurs more readily at lower pressures than at higher ones.

- Freeze drying. In freeze drying, the vapor of water is sublimed off frozen food. This is because under this conditions the food structure is better maintained.

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2.2 Drying Process

There are quite a variety of methods when it comes to drying, depending on the specific requirements we could judge them based on their energy efficiency, time to dry, product quality, among others. (Chen, Mujumdar, 2008), the goal of the drying process is to decrease the weight through the evaporation of moisture from the product, and this should follow one of the trends illustrated in the figure below (see Fig. 3). Basically weight will be decreasing in time.

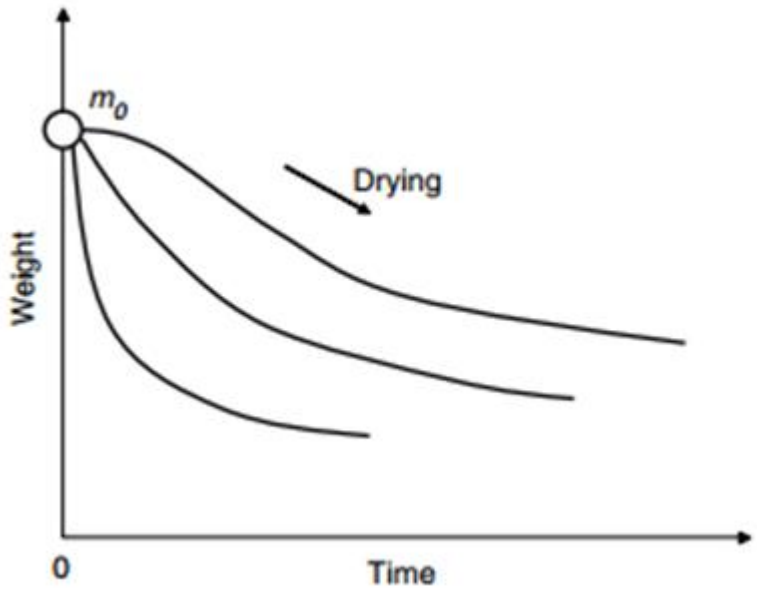

Fig.3 Weight loss curves as drying proceeds (Chen and Mujumdar, 2008)

The drying process not only contributes to microbial growth stability but also modifies the enzymatic reactions and the sensory characteristics of the meat (Grau *et al., 2014*), this will allow extending its shelf life. "Shelf-stable product" refers to those products that do not require refrigeration for freezing for safety and acceptable organoleptic characteristics (Wisconsin University, 2005).

For thermal deactivation of microorganisms, three stages are considered: pre-treatment, heat treatment and post-treatment (Chen and Mujumdar, 2008), each one of them affecting in a different way the drying process. The influence of drying on the microbial activities that are of interest may be classified into two categories: In-drying, which might refer to deactivation of bioactive compounds embedded in liquid food materials, which experience certain temperature-time and moisture content-time profiles during drying. And post –drying usually referred as the deactivation of bioactive compound capture in dried materials which might undergo slow and progressive deterioration depending on different water activity levels (Chen and Mujumdar, 2008).

The main goal of the drying process is to inhibit the growth of microorganism through the evaporation of moisture, is it important to know the minimum water activity required for different microorganisms to grow (see Table 1), thus aside from the drying behavior it's important to identify acceptable levels of water activity to assure proper conservation of products.

Table 1. Minimum water activity level for growth of food-related microorganisms (modified from Gibbs and Gekas 2001)

Food Poisoning Organisms	***Minimum aw***	***Food Poisoning Organisms***	***Minimum aw***	
Campylobacter jejuni	0.98	Yersinia enterocolitica	0.96	
Campylobacter coli	0.97	Clostridium perfringens	0.95	
Bacillius Cereus	0.95	Escherichia coli	0.95	
Clostridium botulinum		Salmonella sp.	0.95	
Type A	0.94	Vibrio paraaemolyticus	0.94	
Type B	0.97	Shigella spp.	0.92	
Type E	0.92	Eurotium amstelodami (Fungi)	0.80	(15 °C)
Listeria monocytogenes	0.92		0.75	(30 °C)
Staphylococcus aureus	0.86	Eurotium Chevalieri (Fungi)	0.85	(15 °C)
			0.75	(30 °C)
		Eurotium herbariorum (Fungi)	0.80	(15 °C)
			0.75	(30 C)

2.3 Heat Transfer in Drying

Dehydration involves the simultaneous transfer of heat, mass and momentum in which heat penetrates into the product and moisture are removed by evaporation into an unsaturated gas phase.

Although it is now accepted that in most practical situations of air drying foods the principal rate-determining step is internal mass transfer, there is no agreement on the mechanism of internal moisture movement. In the case of capillary-porous materials such as fruits and vegetables, interstitial spaces, capillaries and gas-filled cavities exist within the food matrix and water transport takes place. (Gavrila *et al.*.2006)

The rates of drying are generally determined by the rates at which heat energy can be transferred to the water or to the ice in order to provide the latent heats, though under some circumstances the rate of mass transfer (removal of the water) can be limiting. All three of the mechanisms by which heat is transferred - conduction, radiation, and convection - may enter into drying. The relative importance of the mechanisms varies from one drying process to another, and very often one mode of heat transfer predominates to such an extent that it governs the overall process. (Earle, 1983)

The mathematical formula that expresses the heat transfer in air drying can be seen on the Equation Bellow (See Eq. 1)

$$q = h_s A(T_a - T_s) \qquad \text{Eq.1}$$

Where q is the heat transfer rate in Js^{-1}, h_s is the surface heat-transfer coefficient $Jm^{-2}\ s^{10}\ C^{-1}$, A is the area through which the heat flow is taking place, m^2, T_a is the air temperature, and T_s is the temperature of the surface which is drying, °C.

2.4 Drying Kinetics

Air drying is one of the most used unit operations in food processing. Simulation or designing the air drying operation requires the mathematical description of food moisture evolution during the process, known as drying kinetics (Bruce and Giner, 1993).

More generally, air drying is considered a simultaneous heat and mass transfer process where water is transferred by diffusion from inside the food material to the air-food interface, and from the interface to the air stream by convection. Heat is transferred by convection from air to the air-food interface and by conduction to the interior of food. These phenomena have been modeled in two general tendencies: detailed and simplified (Zogzas and Maroulis, 1996).

A schematic of air flow on convection ovens can be observed on the picture bellow (see Fig. 3)

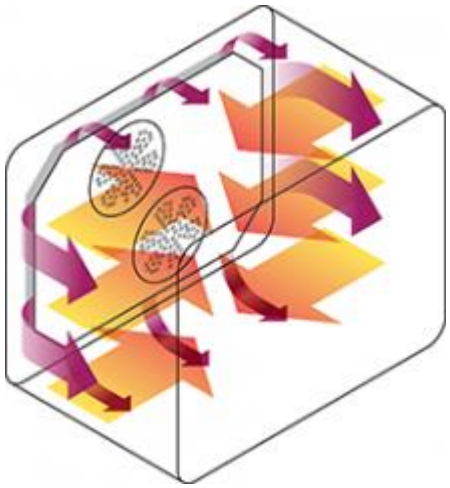

Fig.3 Schematic of Air flow on Convection Ovens (Bertazzoni, 2016)

2.5 Drying Pre- Treatments

Pre-treatment improves nutritional, sensorial and functional properties of the dehydrated food without changing its integrity. It also improves the texture as well as the stability of the pigment during dehydration and the storage of dehydrated product (Wack 1994, Rastogi, 2002).The main goal of drying foods is to extend the shelf life of the product, and to inhibit enzymatic growth as much as possible thus, complementary steps before and after the drying process could be added, and pretreatments are those measures that are taken before the drying process takes place. There are a variety of Pretreatments such as: Blanching Pre-Treatments, Sulfur Dioxide Pre-Treatment, and Dipping Pre-Treatments.

2.5.1 Blanching Pre-Treatments

Blanching is a unit operation prior to freezing, canning, or drying in which fruits or vegetables are heated for the purpose of inactivating enzymes; modifying texture; preserving color, flavor, and nutritional value; and removing trapped air. Hot water and steam are the most commonly used heating media for blanching in industry, but microwave and hot gas blanching have also been studied. Different hot water and steam blanchers have been designed to improve product quality, increase yield, and facilitate processing of goods with different thermal properties and geometries. More recently, energy conservation and waste reduction have driven further improvement of equipment design. Although blanching seems a simple operation, heat transfer to a conveyed bed of product and its effects on product properties are tough to model accurately with predictive mathematics. Processing conditions are usually set up to inactivate enzymes, but other quality parameters, such as color and texture, are commonly monitored. For a given product, typically mass flow rate is fixed, the temperature is measured, and heating media flow rate is adjusted to ensure that the temperature is kept at the set point. (Corcuera et al. 2015)

One of the main disadvantages of the blanching pretreatments is that it might change the texture, color, and flavor and depend on the type of blanching might cause loose of water-soluble vitamins such as C and B.

2.5.1.1 Steam Blanching

In industrial steam blanchers, a product is transported by a chain or belt conveyor through a chamber where ‘‘food-grade’’ steam at approximately 100°C is directly injected. Usually, the temperature in the headspace is measured, and the flow rate of steam is controlled. Steam blanching is generally used for cut and small products, and requires less time than water blanching because the heat transfer coefficient of condensing steam is greater than that of hot water (refer to the articles ‘‘Convection Heat Transfer in Foods’’ and ‘‘Convective Heat Transfer Coefficients’’). However, because of the high-temperature gradients between the surface and the center of the product, larger goods and / or pieces of the product can be ‘‘over blanched’’ near the surface and ‘‘under blanched’’ at the center. To increase heat transfer efficiency, forced convection blanchers have been designed. These blanchers are made of nested chambers that allow recirculating steam with a fan that interconnects both chambers. The fan forces the flow of steam through a packed bed of product conveyed by a mesh belt.

This technology allows higher product bed depths and higher product throughout. Figure 3 shows a picture and a schematic of the cross section of a forced convection blancher. Another technology, individual quick blanching (IQB), was developed to minimize product treatment no uniformities. In IQB, a single layer of product is conveyed through the steam chamber, and each ‘‘individual’’ piece of the product immediately enters into contact with the steam. Steam blanching is more energy-efficient and produces lower biological oxygen demand (BOD) and hydraulic loads than water blanching. In addition, nutrient leaching is reduced compared to water blanching. (Corcuera et al. 2015)

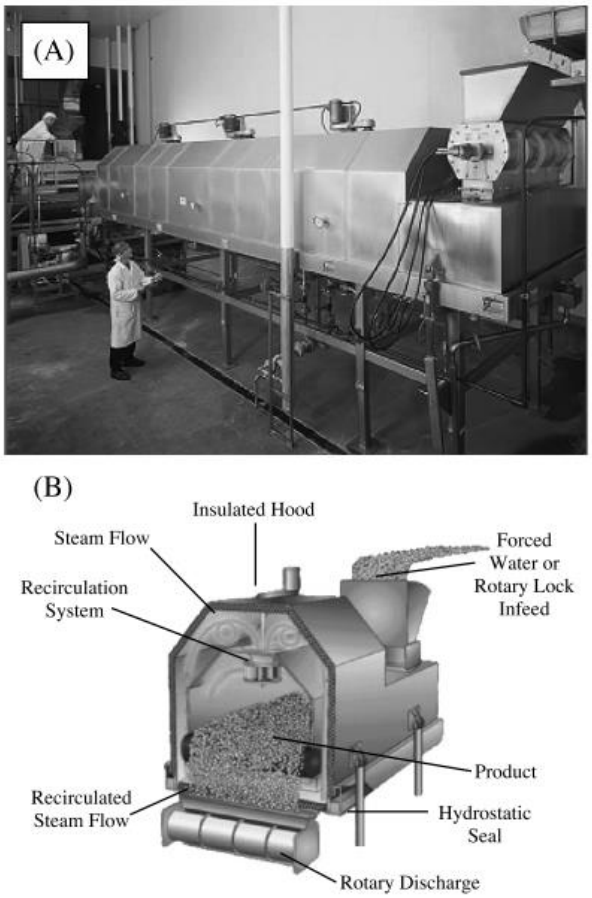

Fig. 3 Forced Convection Blancher (DEKKER, 2015)

2.5.1.2 Water Blanching

Water blanching is performed in hot water at temperatures ranging typically from 70°C to 100°C. However, low-temperature long time (LTLT) blanching and combinations of LTLT with high-temperature short-time (HTST) blanching have also been studied. (Rahman, 1999. Stanley *et al.*, 1995 and Lin *et al.*, 1995). An example can be observed in the figure below (See Fig. 4)

Water blanching usually results in a more uniform treatment, allowing processing at lower temperatures. There are water blanchers that use a screw or a chain conveyor to transport the product inside the tank, where hot water is added. Others use a rotary drum to immerse and convey the product. Water is usually heated indirectly with steam in a heat exchanger; therefore, steam quality does not need to be ‘‘food-grade.’’ Water blanching requires longer processing

times, results in increased leaching of minerals, food, and nutrients such as vitamins, and produces effluents with significant biological oxygen demand (BOD). (Corcuera, 2015)

Fig. 4 Water Blanching

2.5.1.3 Hot Air Blanching (Gas Blanching)

Hot gas blanching using combustion of flue gasses with the addition of steam to increase humidity and prevent product dehydration has been studied. This type of blanching has the advantage of reducing waste production, is comparable to conventional blanching with respect to nutrient retention, but often results in product weight loss. (Corcuera, 2015)

2.5.2 Sulfur Dioxide Pre-Treatment

Sulfur Dioxide is widely used as a pretreatment mostly in fruits; this is due to its properties that help to preserve the texture, flavor, vitamin content and color that make food attractive to the consumer.

Is also widely used in the food industry to reduce the fruit darkening rate during drying and storage, and to preserve and maintain the levels of ascorbic acid and carotene. This pretreatment basically displaces air from the tissue in plant materials, soften cell walls so that drying can occur more easily, destroy those enzymes that cause darkening of cut surfaces, enhance the bright attractive color of dried fruits, this effect can be seen in the figure below (see Fig. 5)

Fig 5. Effects of Sulfur Dioxide Pre-Treatment on Slices of Peach

2.5.3 Dipping Pre-treatment

This pretreatment is usually used in addition to blanching or sulfite treatment; it basically consist n immersion of foods in a solution containing additives. The principal purpose of this treatment is to increase quality and drying characteristics. On the table below it can be seen the different types of chemical used for dipping treatment. (See Table 2)

This kind of pretreatment is mostly used on fruits, as to increase the drying rate, various chemical pretreatments (hot and cold) have been used to increase the drying rate of grapes (Dudman and Grncarevic, 1962; Grncarevic, Radler, and Possingham, 1968; Winkeler *et al.* 1974). For all types of grape drying the dipping pretreatment not only reduces the drying time (makes it more economical), in certain cases it also improves the quality (lighter color, sweeter flavor, nutritional quality and sanitation) of the raisins produced.(Pangavhane *et al.*, 1998).

You have two type of application cold or hot, the treatment with hot dipping solution causes cracking and perforation in the waxy cuticle, increasing the drying rate. However, in cold dipping pretreatment (in which the solution is kept at ambient temperature), though the growth in drying rate is less than that with hot dipping, the raisins produced get an attractive golden brown color without any cracks on the berries. (Riva and Peri,1986)

Table 2. Chemicals Used for Dipping Treatments (Rahman, 1999)

Type	Compounds
Chemicals	
Ester	Methyl oleate, ethyl oleate, butyl oleate
Salts	Potassium Carbonate, sodium carbonate, sodium chloride, potassium sorbate, sodium poly metaphosphate
Organic Acids	Oleic acid, steric acid, caprillic acid, tartaric acid, oleanolic acid.
Oils	Olive Oil
Alkali	Sodium hydroxide
Wetting agents	Pectin, tween, nacconol
Others	Sugar, liquid pectin
Surfactants	
Nonionic	Monoglycerides, diglycerides, alkylated aryl polyester alcohol, polyoxyethylene sorbitan monostearate, D-sorbitol, polyoxyethylene
Anionic	Sodium oleate, stearic acid, sorbitan heptadecanyl sulfate, dimethyl-benzyl-octyl ammonium chloride.

2.6 Role of Antioxidants in Food Preservation

Antioxidants have been used for over 50 years to avoid, or at least delay, the auto-oxidation process (Cuvelier *et al.*, 1994). However, due to concerns about the toxicological safety of synthetic antioxidants such as butyl hydroxyanisole (BHA) and butylhydroxytoluene (BHT) (Branen, 1975), it may be desirable to replace these conventional antioxidants with natural ant oxidative substances. (Mansour and Khalil, 2000)

2.6.1 Artificial Antioxidants

To avoid or delay this autoxidation process antioxidants have been utilized with the practice being carried out successfully for over 50 years (Cuvelier *et al.*, 1994). Synthetic antioxidants

butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are commonly used as food preservatives (Botterweck *et al.* 1990) and are thus consumed in appreciable quantities by humans (Verhagen *et al*., 1990).

Butylated hydroxyanisole (E-320) also known as 2-*tert*-Butyl-4-hydroxyanisole according to its IUPAC name, is perhaps the most extensively used antioxidant in the food industry. BHA can be easily applied to foods because of its excellent solubility in fats and oils. It is heat stable and of all antioxidants it has the best carry-through effect into baked foods, providing extended shelf life (VITABLEND, 2016). Its chemical structure can be seen on the image bellow (see Fig. 5)

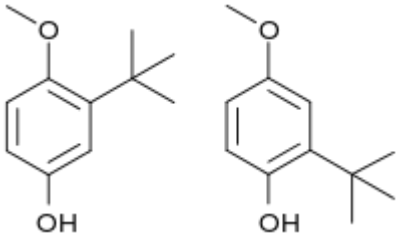

Fig.5 Chemical Structure Butylated hydroxyanisole (BHT)

BHA is one of the primary antioxidants used in feeds because it retards the oxidation of vitamin A, fats, and vegetable oils. It is an effective stabilizer for essential oils, paraffin, and polyethylene (HSDB, 2009). It is used as an antioxidant agent in a biomaterial made from polyurethane and polyethylene oxide used to make mainline catheters (Silverstein *et al*. 1997)
Butylated hydroxytoluene (E-321) also known as 2,6-Bis(1,1-dimethylethyl)-4-methylphenol according to its IUPAC name , is a synthetic analog of vitamin E like BHA and operates by reducing oxygen radicals and interrupting the propagation of oxidation processes. Its volatility at higher temperatures makes it especially suitable for products that are stored at moderate temperatures. (VITABLEND, 2016). Its chemical structure can be seen on the image bellow (see Fig. 7)

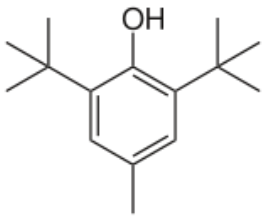

Fig.7 Chemical Structure Butylated hydroxytoluene (BHT)

The chemical properties of both antioxidants can be seen in the table below (see Table 3)

Table 3. Chemical Properties of BHA and BHT (ISDB, 2009 and ChemIDplus, 2009)

Characteristic	**BHA**	**BHT**
Chemical formula	$C_{11}H_{16}O_2$	$C_{15}H_{24}O$
Molar mass	180.25 g·mol^{-1}	220.36 g·mol−1
Appearance	waxy solid	White to yellow powder
Density	1.0587 g/cm^3 at 20 °C	1.048 g/cm3
Melting point	48 to 55 °C (118 to 131 °F; 321 to 328 K)	70 °C (158 °F; 343 K)
Boiling point	264 to 270 °C (507 to 518 °F; 537 to 543 K)	265 °C (509 °F; 538 K)
Solubility in water	Insoluble in water	1.1 mg/L (20 °C)
Solubility	freely soluble in ethanol, methanol, propylene glycol; soluble in fats and oils	
Refractive index(nD)	1.5303 at 589.3nm	

The main concern in the usage of synthetic antioxidants is the potential cancer risk that they carry, BHA and BHT have been classified as reasonably anticipated threat for human carcinogen based on sufficient evidence of carcinogenicity from studies in experimental animals. (NTP, 2014)

Nevertheless, there is not strong proof due to the lack of studies about the correlation between the intake of Synthetic antioxidants and the possibility of development of Cancer, furthermore, A study carried out on population-based nested case-control study of stomach cancer in men and women within the Netherlands Cohort Study of dietary intake found no increase in risk at typical levels of dietary intake of BHA (Botterweck, *et al*. 2000)

2.6.2 Natural Antioxidants

Natural antioxidants are various substances with different chemical characteristics, which are widely present in plants. Antioxidants retard or inhibit oxidation of other substances by inhibiting the initiation or propagation of oxidizing chain reactions (Velioglu *et al.*, 1998). Consequently, natural antioxidants can protect the biologically relevant cellular components from oxidative processes caused by reactive oxygen species (ROS) (Su *et al.*, 2007).

Due to the raising concern about synthetic antioxidants and their potential effects on humans, the usage of more natural alternatives has become imperative, thus, the usage of Natural Antioxidants as replacement of the more commonly used synthetic ones to delay oxidative degradation of lipids, improve quality and nutritional value of foods, have become a critical topic. (Fasseas *et al.*, 2007; Wojdylo *et al.*, 2007; Camo *et al.*, 2008).

Including antioxidants in the diet has beneficial effects on human health because they protect the biologically important cellular components, such as DNA, proteins, and membrane lipids, from reactive oxygen species (ROS) attacks (Su *et al.*, 2007). Synthetic antioxidants have been used to retard or minimize oxidative deterioration of foods, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary butyl hydroquinone (TBHQ). (Fasseas *et al.*, 2007)

2.6.3 Essential Oils

Essential oils are aromatic and volatile liquids extracted from plant material, such as flowers, roots, bark, leaves, seeds, peel, fruits, wood, and whole plant (Deans and Ritchie, 1987; Hammer *et al.*, 1999; Sánchez, *et al.*, 2010). Essential oils have been used for centuries in medicine, perfumery, cosmetic, 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 flavor ingredients increased and became their major employment. Almost 3000 different essential oils are known, and 300 are used commercially in the flavor and fragrances market (Burt, 2004).

Essential oils are considered to be secondary metabolites and important for plant defense as they often possess antimicrobial properties (Fraenkel, 1959; Tajkarimi *et al.*, 2010). The antibacterial properties of secondary metabolites were first evaluated using essential oil vapors by De la Croix in 1881 (Burt, 2004).

Nowadays food industry uses essential oils mostly as flavorings. Nevertheless, they represent a nice source of natural antimicrobials for food preservation. It has to be highlighted that the application of the Essential Oils as food preservatives requires certain knowledge about their properties such as minimum inhibitory concentration (MIC).

Plants produce a variety of compounds with antimicrobial activity. Some are always present while others are produced in response to microbial invasion or physical injury (Roller, 2003).

Some of the most important compounds of the Essential Oils Structure are:

- P-Cymene: The carvacrol precursor p-cymene is a monoterpene that has a benzene ring without any functional groups on its side chains. p-Cymene is not an efficient antimicrobial compound when used alone (Juven *et al.*, 1994)
- Terpenes: Terpenes are hydrocarbons produced from a combination of several isoprene units (C5H8). Terpenes are synthesized in the cytoplasm of plant cells, and the synthesis proceeds via the mevalonic acid pathway starting from acetyl-CoA.
- Terpenoids: Terpenoids are terpenes that undergo biochemical modifications via enzymes that add oxygen molecules and move or remove methyl groups (Caballero *et al.*, 2003).

The chemical structures of this compounds can be seen in the image below (See Fig.8)

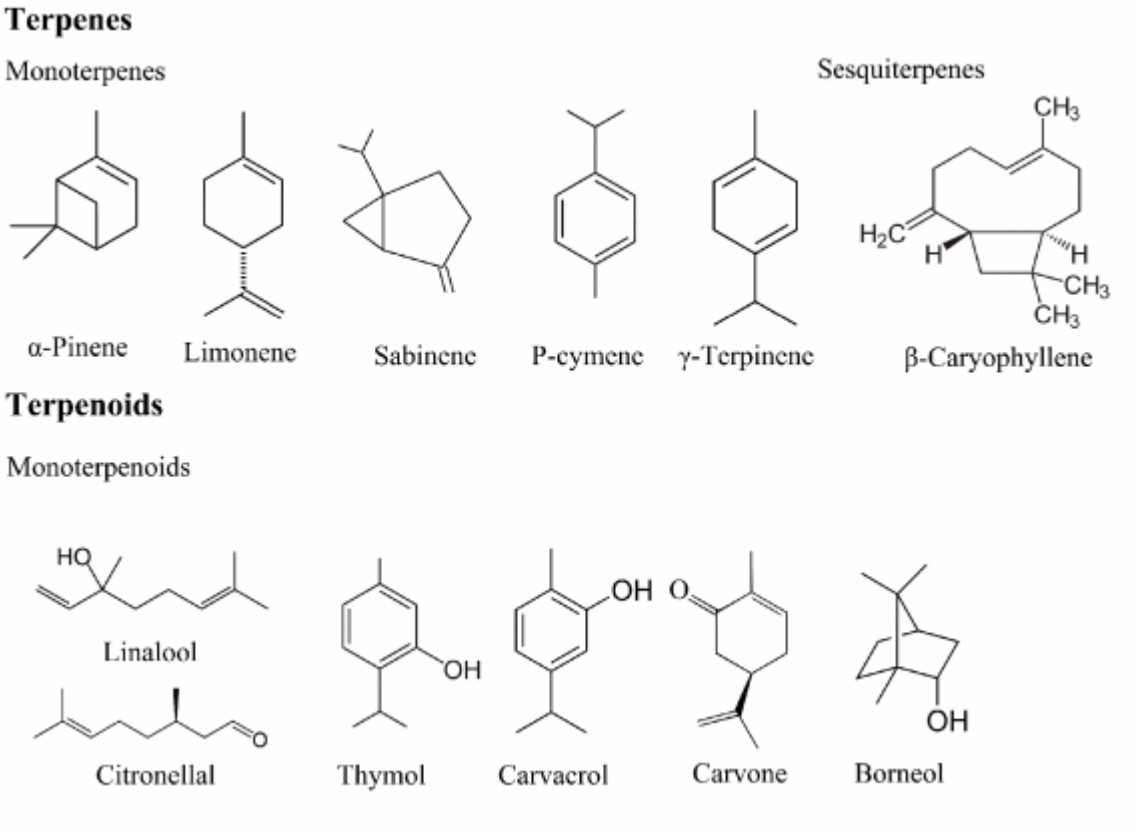

Fig.8 Chemical Structure of Terpenes and Terpenoids compounds (Hyldgaard *et al., 2012)*

On the Table below it's presented some antioxidant capacity of the most important extracts of Essential Oils and its effect on meat after 7 days of storage under refrigeration (see Table 4).

Table 4. Antioxidant capacity and total phenolic compound content of some methanolic plant extracts and their effects on meat quality during 7-d refrigeration. (modified Velasco and Williams, 2011)

Family, scientific name and common name	Antioxidant capacity			Total phenolic content	Meat Characteristics		
	ABTS	DPPH	FRAP		Lipid Oxidation	Color	Antimicrobial Activity
		µM TE 100 g-1 DW		mg GAE 100 g-1 DW	TBA value and mg MDA kg-1		
Lamiaceae Salvia officinalis L. Common name: Sage	17.0 ± 0.23(12)	41.2 ± 1.11(12)	167 ± 1.01(12)	8.25 ± 0.09(12) 1.34 ± 0.09**(3)	TBA < 0.3(10) < 0.5(4) MDA < 0.21(1)		
Origanum vulgare L. Common name: Oregano	19.9 ± 1.00(12)	79.6 ± 2.04(12)	405 ± 2.22(12)	0.15 ± 0.01(12) 11.80 ± 0.60**(3)	MDA < 0.06(14) MDA < 0.4(5, 9)	a* > 12(13) MMG < 30(13)	< 3+(13) < 6(15) < 6.9(2, 9)
Rosmarinus officinalis L. Common name: Rosemary	38.7 ± 0.11(12)	513 ± 5.99(12)	662 ± 4.66(12)	1.71 ± 0.02(12) 2.19 ± 0.15**(3)	MDA < 0.19(1) MDA < 0.5(6) < 0.6(8) MDA < 1(13) TBA < 0.6(4)	a* > 11(13) a* > 17(8) > 20(6) MMG < 20(6, 8, 13)	< 3+(13) < 4(6)
Thymus vulgaris L. Common name: Thyme	35.4 ± 0.12(12)	295 ± 5.83(12)	693 ± 5.87(12)	0.58 ± 0.02(12) 2.13 ± 0.11**(3)	TBA < 0.7(4)		
Lauraceae Cinnamomum zeylanicum Blume Common name: Cinnamon	140 ± 3.01(12) 1064 ± 12.73*(11)	253 ± 3.56(12)	233 ± 2.10(12)	0.13 ± 0.01(12) 14.82 ± 0.28**(11)	TBA < 0.8(4)		

TE: Trolox equivalents; DW: dry weight; ABTS: scavenging of radical 2,2'-azino-bis(3-ethylbenzothiazoline)-6-sulfonic acid; DPPH: scavenging of radical 2,2-diphenyl-1-picryhydrazyl; FRAP: ferric reducing antioxidant power; GAE: gallic acid equivalents; TBA: 2-thiobarbituric acid; MDA: malondialdehyde; MMG: metmyoglobin; CFU: colony-forming unit. *µM g-1 of fresh weight; **mg g-1 of fresh weight; + psychrotrophic aerobic counts.

3. Aim of Thesis

The Aim of this thesis is to establish the effects of oregano essential oil through drying dehydrated meat with the help of three pre-treatments. It will be obtained by comparing drying curves of three pre-treatments with its respective concentration of oregano essential oil (OEO). Moreover, its behavior will be analyzed, in order to identify potential differences among them. The results of comparing will be available for future researchers which are interested in the usage of essential oil for drying meat purposes.

4. Methods and Materials

4.1 Sample preparation

Fresh beef was purchased at a local butchery in Prague to be used in the experiments. It was stored in a freezer at a temperature of -4 degrees. The frozen meat was thawed at 4 degrees overnight, sliced at 0.5 cm thickness with a food Slicer (Concept KP 3530, FS-82T), during the cutting process the samples were done as close to each other as possible in rectangular shape, and later they were stored in pairs in little plastic bags at -4 degrees to be used as needed throughout the experiments. The pieces which are already cut, can be seen in the picture below. (See. Fig. 9)

Fig. 9 Sample beef pieces used in the experiments

Oregano Essential Oil (OEO) was locally purchased through commercial vendor Biomedica (Prague, CZ), the composition of this was 64.5% carvacrol, 5.2%p-cymene, and 2.9% thymol), was acquired 1 kg with CAS 8007-11-2 and EC 290-371-7, the Essential Oil used, in Figure 11 is presented the OEO used during experiments (see. Fig. 10).

Fig. 10 Oregano Essential Oil used in Experiments.

4.2 Drying Pre- Treatments

4.2.1 Hot Air Blanching (HAB)

The samples were thawed at 4°C overnight, once they have been fully unfrozen a piece of filter paper with approximate measurements of 10cmx5cm was cut, later the Oregano Essential Oil was applied using a pipette (Eppendorf 100-1000µl), after being applied the impregnated filter paper was put inside convection oven (Mermet Dryer UFE 400) along with the sample piece of meat for a period of 10 minutes, finishing the pretreatment process.

4.2.2 Steam Blanching (SB)

The samples were thawed at 4°C overnight, once they have been fully unfrozen in a cooking pan aside 1000ml of distilled water were boiled, once water started boiling using a pipette (Eppendorf 100-1000µl) the required concertation of Essential Oil was added, and immediately after it using the vapor produced from the boiling process the sample piece is cooked for 2 minutes, later on, the samples are cooled down with ice for 5 minutes finishing the pretreatment process.

4.2.3 Oil Treatment (OT)

The samples were thawed at 4°C overnight, once they were completely unfrozen with the help of an Erlen Meyer and a pipette (Eppendorf 100-1000µl) 20ml of cooking sunflower oil in combination with the require concentration of Essential Oil are mixed to which afterwards, the meat sample is dipped for 10 minutes and afterwards, any excesses on the sample piece are dried with filter paper, finishing the pretreatment process.

4.3 Instrumentation

Mermert Dryer UFE 400 Convection Owen used for the pretreatment of Hot Air Blanching and for dry matter calculation, it could heat up to +300°C, up to 1060 liters of volume with the option of convection or forced air circulation (see Fig. 11)

Fig. 11 Memert Dryer UFE 400

Climate Box Main equipment used for the drying process, it was set up to a temperature of 60°C to carry out the drying for 6 hours. It has a Temperature range from 25°C – 60°C , internal software for the control of temperature and Relative Humidity, quality control protocol. A climate box KKP115 was used in combination with precision balances RADWAG PS1200 in order to capture the data to the computer as it can be seen on the picture bellow. (See Fig. 12)

Fig. 12 Climate Box KKP 115 with precision balances

Statistics Software for the processing and later statistical analysis of the data the software STATISTICA 10 was used, to carry out the ANOVA test in combination with Tukey test.

4.4 Water Content Calculation

Moisture content analysis is critical component of material quality and essentially a function of quality control in most production and laboratory facilities, from biological research organization, pharmaceutical manufacturers to food producers and packers, moisture content control greatly influences the physical properties and product quality of nearly all substances and materials at all stages of processing and final product existence. (Richardson, 1996)

The moisture content can be:

- Dry Basis Moisture Content (μd)

Dry Basis Moisture content is described by the percentage equivalent of the ratio of the weight of water to the weight of dry matter, is important to highlight that the dry basis moisture content can vary from 0 to vast numbers.

Dry basis moisture is most commonly used for describing moisture changes during drying. When a sample loses or gains moisture, the change in the dry basis moisture is linearly related to the weight loss or gain.

- Wet Basis Moisture Content (μw)

The wet basis is the ratio of the weight of water to the total weight of the material; this measure varies from 0 to 100 percent.

Wet basis moisture is used to describe the moisture content of agricultural materials and food products. When the term "moisture content" is used in the food industry it almost always refers to wet basis moisture content

4.4.1 Evaporation Method

Most methods are based on measuring the mass of water in a known mass of the sample. The moisture content is then calculated by a simple measure of the weight before and after the drying process while the water is removed by evaporation.

Moisture content in food material is defined by the expression shown below (see Eq. 2)

$$\%\ Moisture = \frac{\mu_f - \mu_o}{\mu_o} x\ 100\% \qquad \text{Eq. 2}$$

Where:

μ_o = Initial mass of the sample

μ_f = Final mass of the sample

The type used on the experiments was the Convection and force draft oven method, we dried the samples for 24 hours at 105°C (Kucerova, 2015), the process consist on first putting the weighted sample on the oven for the specified amount of time and temperature then the dried mass it determined as they are dried until they reach the constant mass. (Oluwatosin, 2005).

Then we calculate Moisture Ratio (MR) which allows to obtain the curves, Moisture content dry basis (μd) is used and then converted to Moisture Ratio to have the curves in a scale from 0 to 1 which will make easier to appreciate any significant difference. Below it can be seen the formula through which we calculate Moisture Ratio (see Eq.3)

$$MR = \frac{\mu d_i}{\mu d_1} \qquad \text{Eq.3}$$

Where μd_i is the moisture content dry basis at a given point "i" and μd_1 is the first measurement of Moisture content dry basis.

4.5 Experimental Process

Once samples were prepared and frozen, twenty four hours before every experiment a plastic bag with the beef samples was unfrozen at 4°, this allowed the sample piece to be ready for the pre-treatment.

Before starting any experiment the Climate box (POLEKO KKP115) was started before the drying process in order to achieve pre-established conditions of temperature at 60°C, this will guarantee stable temperature level during the 6 hours of drying.

Following with the process each sample was weighted and submitted to the respective pre-treatment and concentration of Oregano Essential Oil (OEO) which were defined to be 1.5ml, 3ml and 6ml, for every concentration and pre-treatment respectively each experiment was repeated three times.

Immediately after the pre-treatment the sample piece was put to dry on the climate box (POLEKO KKP115) for a period of 6 hours along with a control piece that was not previously submitted to any type of pretreatment, during this 6 hours and with the help of the precision balance (RADWAG PS1200 R2) and the computer software (Pomiar-win v.5.2.0) a sample of the weight for both the sample piece and the control was taken every minute, this was later graphed and analyze with the statistics software (Statistica V10.0).

After the 6 hours of drying and having collected all the data on the computer, the sample was later dry to 105° for 24 hours in a convection oven (Memert UFE 4.0) to obtain the dry matter content to be able to calculate the Moisture Content on a dry basis, in order to proceed with the analysis.

4.6 Statistical Analysis

During the statistical analysis due to the high amount of data that was going to be processed and the two combinations to be taken on to consideration pre-treatment and concentrations a combination approach between ANOVA multifactorial with Tukey Honest Significant Difference Test was used, with a confidence level of $\alpha=0.95$

4.6.1 ANOVA Multifactorial

Anova is statistical analysis method that stands for analysis of variance and is an extension of T-test and z-test. The use of ANOVA depends commonly on the research design for our case we used the multifactorial ANOVA which consist on the comparision of more than two groubs based on two or more factors (independt variables), as it could be type of pre-treatment and concentration of EOE for instance.

Multifactorial ANOVA's can be used to see the effect of one or more factors over the other ones, or it can be used to see the interaction between two factors.

Factorial ANOVA can be balanced or unbalances; this is to say you can have the same number of subjects in each group (balanced) or not (unbalanced). This can come about, depending on the study, as just a reflection of the populations (STATISTIC SOLUTIONS, 2014).

4.6.2 Tukey's test

The Tukey is a post hoc test that can be used in combination with the ANOVA Test, to find means that are significantly difference from each other, it basically compares all possibilities from a pair of means and is based on the studentized range distribution (q).

It basically compares the mean of each test to each average of every other treatment to set all pairwise comparisons while providing narrower results, and it highlights any difference higher than the standard error.

Tukey test is essentially a T-test with the difference that this one corrects for experiments wise error rate, thus makes it more suitable for multiple comparison than doing a number of t-tests will be.

The formula for Tukey test can be seen bellow (see Eq. 3):

$$q_s = \frac{Y_a - Y_b}{SE} \qquad \text{Eq. 4}$$

5. Results and Discussions

5.1 Influence of drying pre-treatment on drying kinetics

5.1.1 Dipping Oil Treatment (OT).

The higher concentrations of oregano essential oil (OEO) during the pre-treatment shows a speed up in the drying process compared to lower concentrations (see Fig. 13). Furthermore, compared with the control samples, it affected drying by making it slow, this happened probably due to the coating of the surface by wax-like oil. Most likely, this in combination with the hydrophobicity of the mix of oregano and sunflower oil, has influenced reduction of the ratio of moisture evaporation.

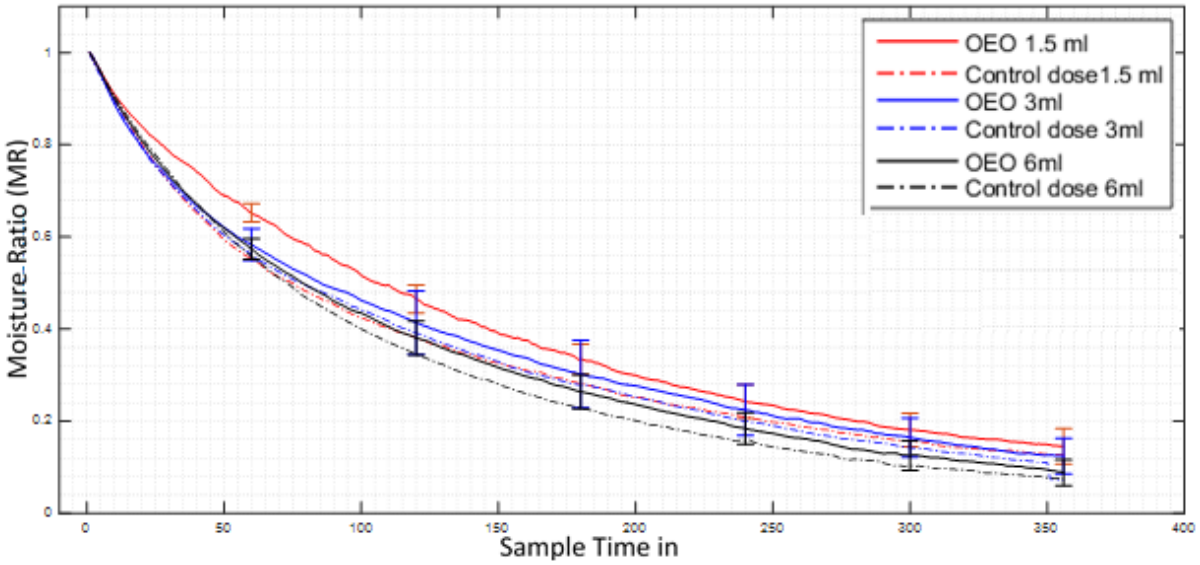

Fig. 13 Moisture ratio graph oil pre-treatment (OT) all concentrations

Similar effect was observed on the moisture movement during the drying of seedless grapes (Pangavhane *et al.*, 1999) and on bell pepper (Tunde-Akintunde *et al*., 2011). Once the statistical analysis was carried out, it could be observed that the difference reflected on the graph is statistically significant ($p<0.05$) only for the case of the concentration of 1.5ml. The table can be seen below (see Table 5)

This could be explained since at lower concentration of OEO there is then more sunflower oil available to create the wax-like layer which is resulting in slowing down the overall drying process. What is more, the higher the concentration, the less significant is the difference.

In the statistical analysis it was confirmed that the OT has its limitations when it comes to speeding up the drying process. Since it can be observed that the statistical significance ($p<0.05$) is only seen in comparison between the smaller dose of 1.5ml vs 3ml and 1.5ml vs 6ml, but it cannot be seen in comparison between 3ml and 6ml dose, confirming such limitation.

Table 5. ANOVA Factorial Statistical Test, Oil Pre-treatment Different Concentrations

Type of Pre Treatment	Type of Concentration	OT C1	OT C2	OT C3	COT C1	COT C2	COT C3
OT	C1		**0.01217**	**0.00004**	**0.00006**	**0.00004**	**0.00004**
OT	C2	**0.01217**		0.17420	0.96280	0.75453	**0.00004**
OT	C3	**0.00004**	0.17420		0.99994	0.99999	0.44664
COT	C1	**0.00006**	0.96280	0.99994		1.00000	0.07829
COT	C2	**0.00004**	0.75453	0.99999	1.00000		0.05389
COT	C3	**0.00004**	**0.00004**	0.44664	0.07829	0.05389	

OT: Oil Pre Treatment; COT: Control Pre-Treatment; C1: Concentration of 1.5 ml, C2: Concentration of 3ml; C3: Concentration of 6ml.

5.1.2 Steam Blanching (SB)

The different dose of OEO used does not reflect any difference in the drying process (see Fig.14), this could be the result of a more aggressive action of the pretreatment which will surpass any effect from the essential oil itself.

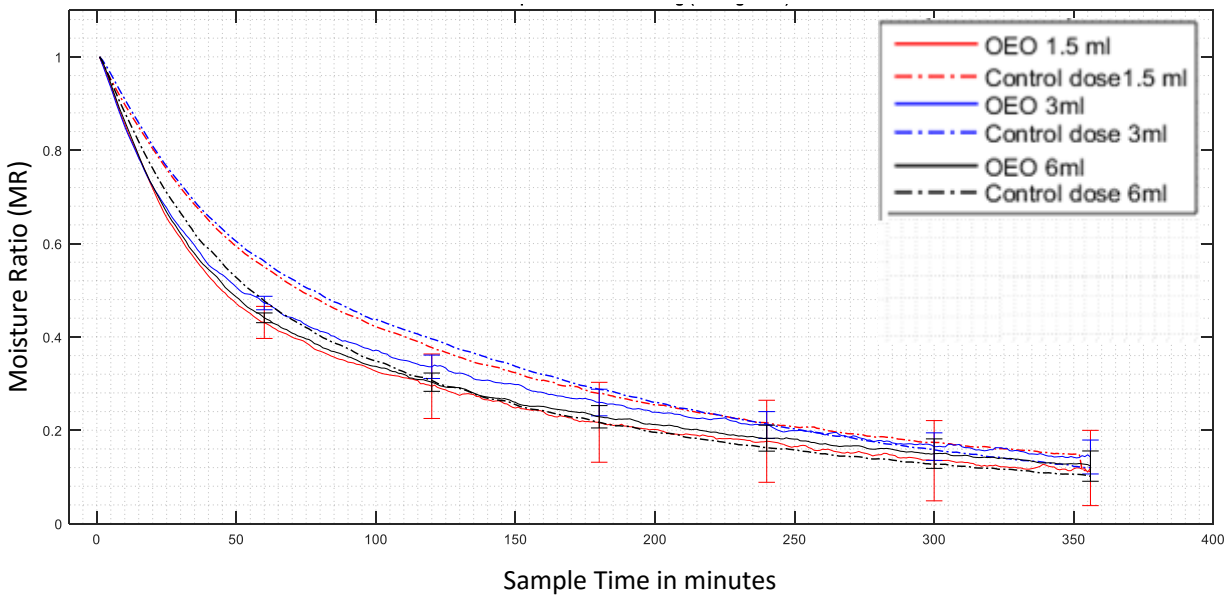

Fig. 14 Moisture ratio graph steam blanching pre-treatment (SB) all concentrations

Furthermore, as it can be seen on the figure above (see Fig. 14), there is a difference between the control samples and those that received the dose of OEO with the help of the pre-treatment. The difference is caused by the structural change of the product sample pieces, which most likely was due to the pores being enlarged as an effect of the blanching process and caused the water to evaporate faster.

These results are similar to those obtained on (Kingsly *et al.*, 2007; Akanbi *et al.*, 2003; Turhan *et al.*, 1997 and Mazza, 1983), which confirms that the effect of the blanching procedure has over the drying time.

Nevertheless, the statistical analysis doesn't show this difference as significant as it can be seen in the table below. ($p>0.05$) (see Table 6)

Table 6. ANOVA Factorial Statistical Test, Steam Blanching Pre-treatment Different Concentrations

Type of Pre Treatment	Type of Concentration	SB C1	SB C2	SB C3	CSB C1	CSB C2	CSB C3
SB	C1		**0.022111**	0.999234	0.000036	0.000036	0.999987
SB	C2	**0.022111**		0.521111	0.083031	0.119094	0.585595
SB	C3	0.999234	0.521111		**0.000036**	0.000042	1.000000
CSB	C1	0.000036	0.083031	**0.000036**		1.000000	**0.000044**
CSB	C2	0.000036	0.119094	0.000042	1.000000		0.000072
CSB	C3	0.999987	0.585595	1.000000	**0.000044**	0.000072	

SB: Steam Blanching Pre Treatment; CSB: Control Steam Blanching Pre-Treatment; C1: Concentration of 1.5 ml, C2: Concentration of 3ml; C3: Concentration of 6ml.

5.1.3 Hot Air Blanching (HAB)

In a same way as a blanching pre-treatment works, same results should be obtained from the SB pre-treatment. Nevertheless, due to the less aggressive approach, no potential difference can be seen from different concentrations used neither with the control meat samples, which is a direct consequence of the lower temperature of blanching compared to those achieved with steam blanching. (see Fig.15)

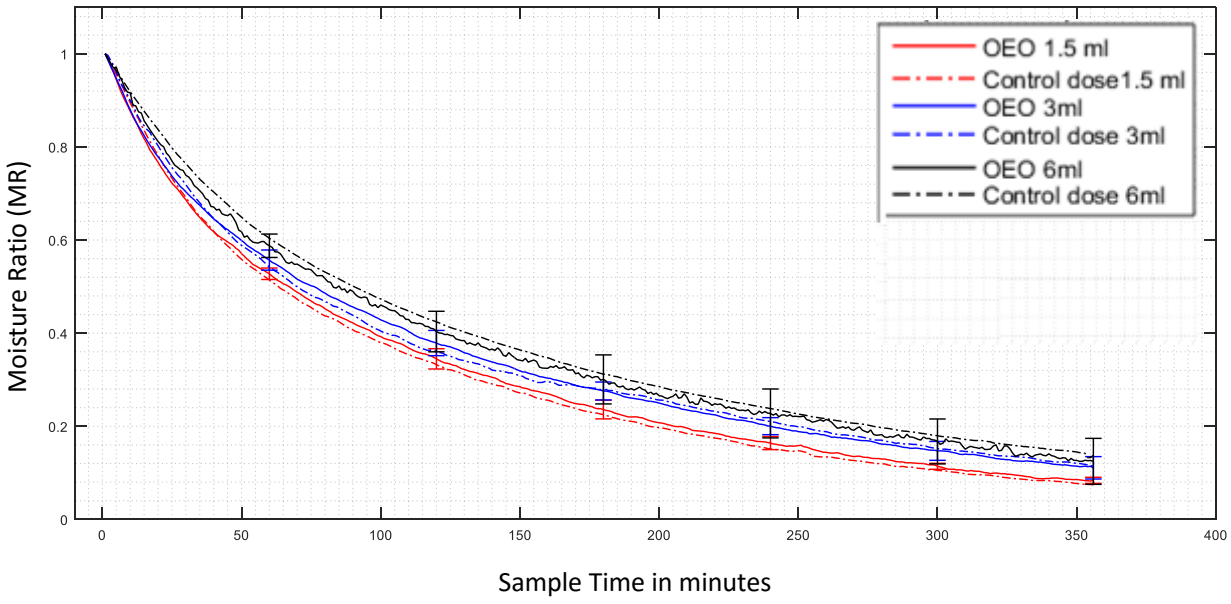

Fig. 15 Moisture ratio graph hot air blanching pre-treatment (HAB) all concentrations

On an opposite outcome from steam blanching, no difference could be observed between the treated samples and their controls; this reaffirms the less aggressive nature of the blanching pre-treatment. Furthermore, no difference can be observed compared to their respective controls, see figure above. (see Fig. 15)

In the statistical analysis, it is confirmed that there is no statistical significance difference ($p>0.05$) among the treated samples with different doses neither with their respective controls, affirming the passive effect of the pretreatment over the meat sample. (see Table 7)

Table 7. ANOVA Factorial Statistical Test, Hot Air Blanching Pre-treatment Different Concentrations

Type of Pre Treatment	Type of Concentration	HAB C1	HAB C2	HAB C3	CHAB C1	CHAB C2	CHAB C3
HAB	C1		0.047414	**0.000036**	0.999986	0.569982	**0.000036**
HAB	C2	0.047414		0.441856	**0.001394**	1.000000	**0.001959**
HAB	C3	**0.000036**	0.441856		**0.000036**	0.919952	0.962625
CHAB	C1	0.999986	**0.001394**	**0.000036**		0.158405	**0.000036**
CHAB	C2	0.569982	1.000000	0.919952	0.158405		0.156739
CHAB	C3	**0.000036**	**0.001959**	0.962625	**0.000036**	0.156739	

HAB: Hot Air Blanching Pre Treatment; CHAB: Control Hot Air Blanching Pre-Treatment; C1: Concentration of 1.5 ml, C2: Concentration of 3ml; C3: Concentration of 6ml.

5.2 Influence of oregano essential oil on drying kinetics

5.2.1 Concentration of 1.5ml of OEO

Confirming the previously found results it could be seen that OT treatment is the slowest of all and moreover, that the HAB pre-treatment is softer than SB (see Fig.16). This happens due to the more aggressive approach from the SB which is causing the opening of the pores and allowing moisture to go out faster. These results are in accordance with those obtained by (Tunde-Akintunde *et al*., 2011).

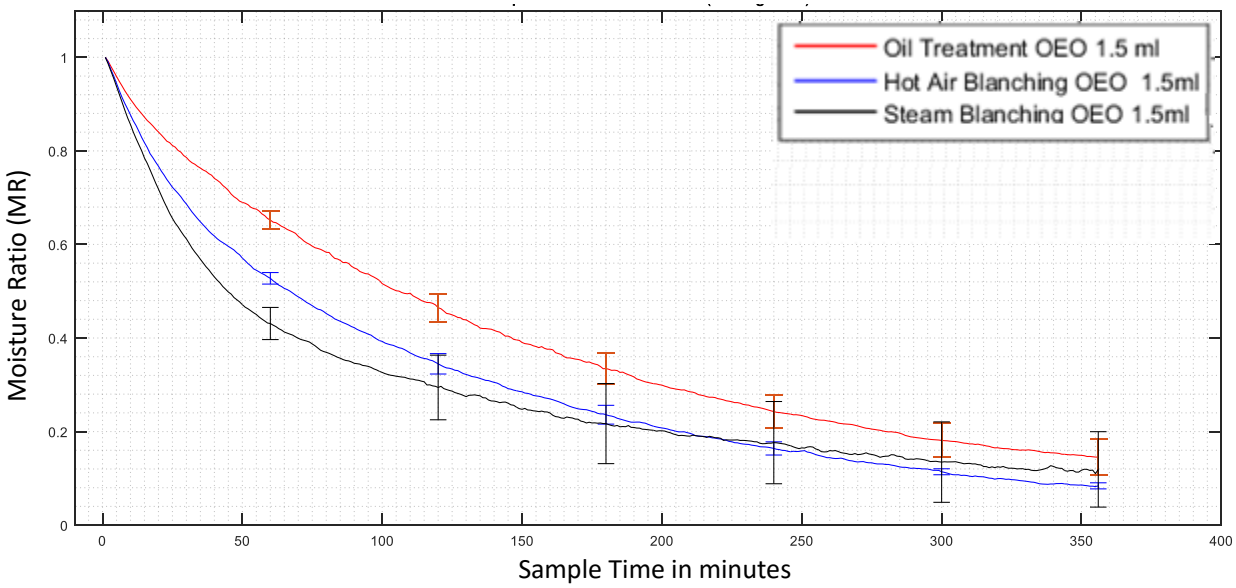

Fig. 16 Moisture Ratio All Pre Treatments Dose of 1.5ml

In the statistical analysis it can be observed that the only statistical significant difference ($p<0.05$) between OT and SB and HAB is due to the effect of making the drying slower, see on the table below (see Table 8.)

Table 8. ANOVA Factorial Statistical Test, for concentration of 1.5ml

Type of Pre Treatment	Type of Concentration	OT	HAB	SB
OT	C1		**0.000036**	**0.000036**
HAB	C1	**0.000036**		0.586867
SB	C1	**0.000036**	0.586867	

OT: Oil Pre Treatment, HAB: Hot Air Blanching Pre Treatment; SB: Steam Blanching Pre-Treatment; C1: Concentration of 1.5 ml.

5.2.2 Concentration of 3ml of OEO

The order of the curves by pretreatment is the same as from 1.5ml dose having the OT as the slowest and SB as the fastest. Nevertheless, on the figure below (see Fig.17) it can observed that the difference between OT and SB is not as big as the one with 1.5ml. This confirms that the correlation between high concentration and fast drying during the OT pre-treatment. Furthermore, it also confirms that this difference is every time less significant with higher doses of OEO, which is due to the more diluted mix of sun flower and oregano essential oil making the coating less effective which results in drying faster.

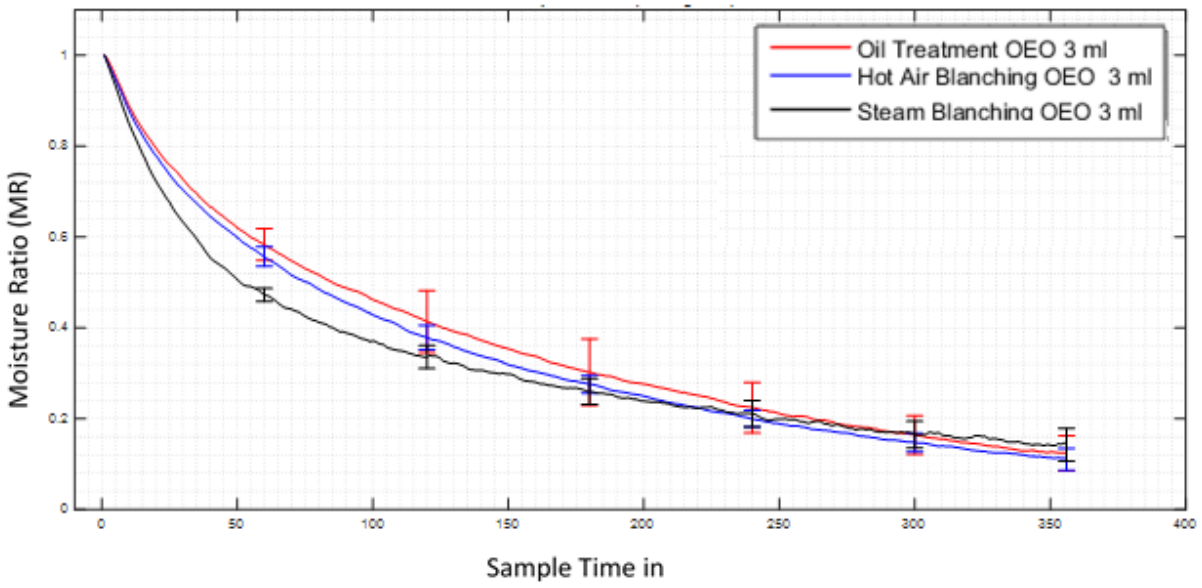

Fig. 17 Moisture Ratio All Pre Treatments Concentration of 3ml

This is confirmed by the statistical analysis reflecting no longer statistical significance difference ($p>0.05$) between the OT treatment and its counterparts of SB and HAB, which can be seen on the table below (see Table 9).

Table 9. ANOVA Factorial Statistical Test, for concentration of 3ml

Type of Pre Treatment	Type of Concentration	OT	HAB	SB
OT	C2		0.457271	**0.000254**
HAB	C2	0.457271		0.751380
SB	C2	**0.000254**	0.751380	

OT: Oil Pre Treatment, HAB: Hot Air Blanching Pre Treatment; SB: Steam Blanching Pre-Treatment; C1: Concentration of 3 ml.

5.2.3 Concentration of 6ml of OEO

On the contrary to the pattern observed for the dose of 1.5ml and 3ml, the order of the pre-treatments have changed, being now the slowest one the HAB, once again reaffirming that the higher the concentration on the OT the drying takes place faster, and on this case as it can be seen on the figure below (see Fig. 18), compared to SB, the OT pre-treatment has indeed became faster as direct effect of the OEO probably due to the effect over the coating as a result of the pretreatment.

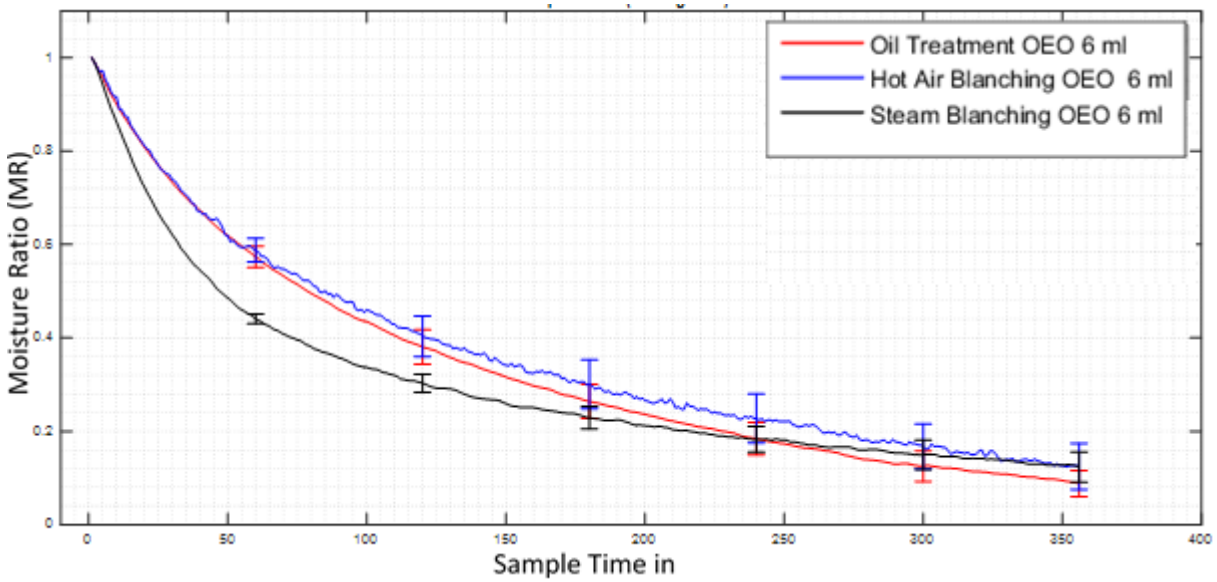

Fig. 18 Moisture Ratio All Pre Treatments Concentration of 6ml

On the statistical Analysis it can be seen that besides the statistical significance difference ($p<0.05$) of SB pre-treatment there is no statistical significance among the other pretreatments (see Table 10), meaning that despite the improvement of the OT the difference with SB is still significant enough to confirm that SB is the fastest treatment of all three.

Table 10. ANOVA Factorial Statistical Test, for concentration of 6ml

Type of Pre Treatment	Type of Concentration	OT	HAB	SB
OT	C3		0.165374	**0.002798**
HAB	C3	0.165374		**0.000036**
SB	C3	**0.002798**	**0.000036**	

OT: Oil Pre Treatment, HAB: Hot Air Blanching Pre Treatment; SB: Steam Blanching Pre-Treatment; C1: concentration of 6 ml.

6. Conclusions

Having highlighted the importance of the meat consumption, which is taking over the incoming years, and also having identified the major cons of the currently widely used artificial antioxidants, allowed us to focus this research on the more healthy alternatives of the natural antioxidants and the so called essential oils, more specifically oregano essential oil.

Moreover, having no previous studies over the effects of essential oil usage on drying kinetics of dehydrated meat, focusing the aim of the thesis on doing this analysis focused on oregano essential oil was a really innovating approach. With the help of three pre-treatments and the proper acquisition of the drying curves in combination with an accurate statistical analysis it has allowed not only to conclude interesting findings on the topic, but also has allowed us to provide a discussion for future researchers which are interested in the usage of essential oil for drying meat purposes.

For this purpose our conclusions were:

- The only pre-treatment showing some correlation with the doses of OEO applied is the OT pre-treatment, probably is due to the diluting effect over the mixture of Sunflower and Oregano Essential Oil and its direct effect on the coating to which the meat samples were subject to, allowing to moisture to evaporate faster.

- It can be concluded that on the SB process the fastest drying is a direct effect of the pre-treatment rather than from EOE effect, confirmed by the lack of statistical significance difference ($p>0.05$) among the treated samples, which is probably due to the aggressive approach of steam cooking directly related to high temperatures, which directly opens the pores of the product allowing faster evaporation of moisture.

- HAB is the most passive pre-treatment of all three, since it did not reflect any significant effect over the treated samples, which is because of the blanching taking place through vapor rather than steam in combination with lower blanching temperatures (blanching

pre-treatment takes place at 55°C), having no statistically significant difference ($p>0.05$) between the different doses of OEO nor the treated samples and their respective controls.

- It can be concluded that on the OT pretreatment that the higher the dose applied is, the less significance the difference is, reflecting it in a way that the capacity of speeding up the drying process through the usage of higher concentration of EOE has its limitation, and it is decreasing with higher dose of OEO.

- It can be concluded that no evidence was found that higher concentrations of OEO will do a significance difference, besides the difference on speed obtained during the increasing the concentration from 1.5ml to 3ml on the OT treatment, there was no evidence found that increasing the dose of OEO will affect the drying speed of the product.

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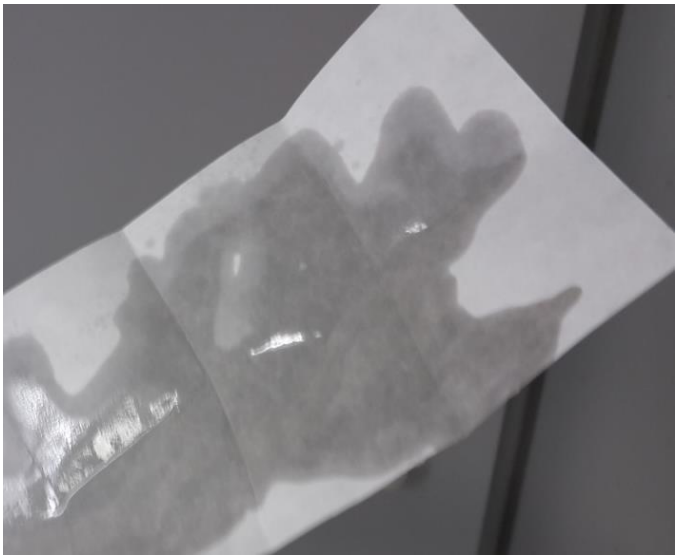
Appendix A.

Photo documentation of Experiments.

a. Piece of fresh beef locally acquired at a butchery in Prague.

b. Sample pieces already cut.

c. Filter paper with OEO dose already applied for HAB treatment.



d. Sample Piece been applied HAB treatment on Convection Oven.

e. Convection Oven memmert dryer used on the experiments.

f. Climate box with two precision balances adapted to take measurements for drying curves.

g. Meat samples inside climate box being dried.